ISOLATION, SPECIATION AND ANTI FUNGAL SUSCEPTIBILITY OF DERMATOPHYTIC INFECTION IN PATIENTS ATTENDING A TERTIARY CARE HOSPITAL

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CERTIFICATE

This is to certify that this dissertation entitled **"ISOLATION, SPECIATION AND ANTI FUNGAL SUSCEPTIBILITY OF DERMATOPHYTIC INFECTION IN PATIENTS ATTENDING A TERTIARY CARE HOSPITAL"** is the bonafide original work done by **Dr.S. VANATHI,** Post graduate in Microbiology, under my overall supervision and guidance in the Department of Microbiology, Govt. Kilpauk Medical College, Chennai, in partial fulfillment of the regulations of The Tamil Nadu Dr. M.G.R. Medical University for the award of **M.D Degree in Microbiology (Branch IV).**

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This is to certify that the dissertation entitled **"ISOLATION, SPECIATION AND ANTI FUNGAL SUSCEPTIBILITY OF DERMATOPHYTIC INFECTION IN PATIENTS ATTENDING A TERTIARY CARE HOSPITAL"** is a bonafide research work done by **Dr. S. VANATHI,** Post graduate in Microbiology, under my guidance in the Department of Microbiology, Govt. Kilpauk Medical College, Chennai, in partial fulfillment of the regulations of The Tamil Nadu Dr. M.G.R. Medical University for the award of **M.D Degree in Microbiology (Branch IV).**

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DECLARATION

I solemnly declare that this dissertation "**ISOLATION, SPECIATION AND ANTI FUNGAL SUSCEPTIBILITY OF DERMATOPHYTIC INFECTION IN PATIENTS ATTENDING A TERTIARY CARE HOSPITAL**" is the bonafide work done by me at the Department of Microbiology, Govt. Kilpauk Medical College and Hospital, Chennai, under the guidance and supervision of **Dr.RADHIKA KATRAGADDA, M.D.,** Professor & H.O.D of Microbiology, **Dr. THYAGARAJAN RAVINDER, M.D.,** Professor, Department of Microbiology and **Dr. K.V. LEELA, M.D., DGO.** Associate Professor of Microbiology Department of Microbiology, Govt. Kilpauk Medical College, Chennai-600 010. This dissertation is submitted to The Tamil Nadu Dr. M.G.R. Medical University, Chennai in partial fulfillment of the University regulations for the award of Degree of M.D. Branch IV Microbiology examinations to be held in April 2015.

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ABSTRACT

TITLE: ISOLATION, SPECIATION AND ANTI FUNGAL SUSCEPTIBILITY OF DERMATOPHYTIC INFECTION IN PATIENTS ATTENDING A TERTIARY CARE HOSPITAL

INTRODUCTION:

Dermatophytoses are the infection confined to the dead cornified layers such as skin, hair and nail. Dermatophytoses are common in tropical countries like India due to hot and humid climate. Nowadays susceptibility to fungal infection is increasing due to inadvertent use of antibiotics and newer modality of management. Isolation, speciation and antifungal susceptibility pattern aids the clinician to select the appropriate antifungal agent for the management of dermatophytic infection.

AIMS AND OBJECTIVES:

- To isolate the dermatophytes causing infection of skin, hair and nail.
- To identify and speciate the isolates of dematophytes.
- To determine the antifungal susceptibility of the isolates.

MATERIALS AND METHODS:

Specimen collection :

Skin, hair, and nail are taken from active edge of the lesion and are examined in 10% KOH to detect fungal hyphae. All specimens are inoculated in duplicate in Modified Sabouraud ‘s Dextrose agar and Dermatophyte test medium which are incubated at 25 - 37°c for 10 days - 2 weeks .Identification was done on the basis of the colony characteristics and microscopic morphology in lactophenol cotton blue mount .The antifungal susceptibility test was done by micro broth dilution method to detect MIC for Griseofulvin, Ketoconazole, Fluconazole and Itraconazole.

RESULT:

217 clinically suspected cases of dermatophytes were subjected to mycological study. Most of the cases were seen between 21 – 30 years of age group. Male to Female ratio was 1.58 : 1. 80(36.86%) samples were showed culture positive.

Trichophyton species were the predominant isolates (96.2%) followed by Epidermophyton (2.25%) and Microsporum (1.25%) Trichophyton rubrum was the predominant isolate in Tinea corporis, , Tinea cruris, Tinea capitis and Tinea unguium

In vitro hair perforation test and Urease test helps to differentiate Trichophyton mentagrophytes from Trichophyton rubrum. The minimal inhibitory concentration of Griseofulvin was 0.03-0.5µg/ml. The minimal inhibitory concentration of Fluconazole was 1-8 µg/ml. The minimal inhibitory concentration of. Itraconazole was 0.015-0.25 µg/ml.

Fluconazole showed a higher MIC value when compared to other drugs by micro broth dilution method. Itraconazole was found to be the most effective of all the drugs tested.

CONCLUSION:

Totally six species were isolated on culture. Trichophyton rubrum was the predominant isolate followed by Trichophyton mentagrophytes, Trichophyton verrucosam , Trichophyton tonsurans, Epidermophyton flocccosum and Microsporum gypseum DTM are the special medium used for the persumtive diagnosis of dermatophytes. Itraconazole was found to be the most effective of all the drugs tested. Specific identification of the dermatophytic species and timely institution of appropriate antifungal therapy based on the prevailing sensitivity pattern could be of immense value**.**

Key words: Dermatophytoses, Trichophyton, Epidermophyton, Microsporum MIC.

INTRODUCTION

Dermatophytoses are the infections of keratinized tissues such as the epidermis, nail and hair caused by a group of closely associated filamentous fungi called as Dermatophytes [1]. These fungi produce proteases that digest and use keratin as a source of nitrogen and permits infection, colonization and invasion of the stratum corneum of the skin, nail and hair shaft [2,7]. These fungi cannot penetrate the deeper tissue of a healthy immunocompetent host. So, the infection is generally restricted to the dead cornified layers. Tinea in Latin means ringworm.

Depending upon the anatomical site and the etiological agent, Dermatophytes are classified into different varieties .The second part of the name of the dermatophytosis identifies the part of the body infected, i.e, in case of Tineacorporis, "corporis" refers to the body. Dermatophytes are a mold which belong to the fungi imperfecti, has three genera Trichophyton, Epidermophyton and Microsporum.[2,5] Dermatophytes may be grouped into Anthropophilic (human loving), zoophilic (animal loving) and Geophilic (Soil loving). Dermatophytosis does not cause mortality but they cause morbidity.

Dermatophytoses are common in tropical countries like India due to hot and humid climate[4,8,44]. The risk factor include overcrowding, poverty, poor personal hygiene, poor peripheral circulation[5,33], immunosuppressive therapy, cancer chemotherapy and immunocompromised conditions[6,9,10]. Mode of transmission is by contact with the infected person / pet animals, fomites or auto inoculation from another body site[4].

Fungal metabolic products diffuse through the Malphigian layer of the epidermis to cause erythema, vesicles and even pustule formation along with pruritis[2]. The characteristic skin lesion is red and circular scaly patch with sharply demarcated margin. The hyphae become old and break into arthrospores, which are shed off in due course of time which is held responsible for the central clearing of the lesion. The severity of these reactions are related to the involved sites, fungal species , size of inoculum and immune status of the host .Modified Sabourauds dextrose agar with cycloheximide , chloramphenicol and gentamycin is used for the culture of the dermatophytes[3,12,103].

Numerous anti fungal agents have been developed after a breakthrough invention of Griseofulvin by Gentles in guinea pigs in 1958. Later in 1980

invention of ‘azoles’ and associated groups of antifungal drugs were available for the management of dermatophytosis[2,25]. Nowadays susceptibility to fungal infection is increasing due to inadvertent use of antibiotics and newer modality of management. Antifungal susceptibility test helps the clinician to select a specific antifungal agent.

The reference for micro broth dilution method of the filamentous fungi (CLSI M38A) established the interpretive break points for the newer triazoles (posaconazole, ravuconazole, and voriconazole), fluconazole and itraconazole[14,15]. Early diagnosis and initiation of treatment is essential for reducing the emergence of resistant strains and morbidity. This study identifies the species causing dermatophytoses and their antifungal susceptibility.

AIMS AND OBJECTIVES

- To isolate the dermatophytes causing infection of skin, hair and nail.
- To identify and speciate the isolates of dematophytes.
- To determine the antifungal susceptibility of the isolates.

REVIEW OF LITERATURE

Historically Agostino Bassi (1835-36) revealed the microbial character of decaying infection of silkworm (Bombyx mori). He established clearly that Beauveria bassiana a mold was the etiological agent of this disease [2,25]. But in 1873 hyphae in the scalp scrapping was first observed by Robert Remak. In 1839, Professor Johann L Schonlein described these filaments as molds and considered plant as source of infection [2,25].

David Gruby, the Hungarian physician, who was working in Paris, described the clinical entities that favo, the scalp diseases caused by dermotophytes and demonstrated that fungi could be cultured and transmitted to human [2]. Majocchi's granuloma, a variant of Tinea corporis, is an uncommon infection of dermal and subcutaneous tissue caused by dermatophytes which was first described by Domenico Majocchi (1849-1929). In 1883, he named this disorder as Granuloma Tricofitico.

In 1910, a French dermatologist Raymond Sabouraud, published his monumental work, Les Teignes, the comphrehensive account of all the then known dermatophytes to which mycologist still refer[25]. Based on the clinical nature of the disease, cultural & microscopic features, he classified dermatophytes into Trichophyton, Epidermophyton, Microsporum and Achorion[31,37]. In 1925 Robert W.Wood, a Baltimore physician invented the Wood's lamp for detection of fungal infection of hair.

In 1934, Chester Emmons redefined and established the current classification of Dermatophytes on the basis of spore morphology in which he eliminated the genus Achorion and emphasized on the rest of the three genera Epidermophyton, Microsporum and Trichophyton.

In 1959, Dawsan and Gentles using hair bait technique of Vanbreusegham, led to the discovery of Telomorphs of many dermatophytes and related keratinophilic fungi. In 1958, Gentles discovered the antifungal activity of Griseofulvin after his work on guinea pigs[2,25]. In 1980 discovery of azole derivatives and allied group of antifungal drugs had significant impact on the management of dermatophytosis.

3.1 NATURAL HABITAT

Depending on their ecological characteristics, Dermatophytes are classified into geophilic, zoophilic and anthropophilic species. Anthropophilic Dermatophytes are fungal species exclusively affecting human eg-T. rubrum, Trichophyton tonsurans[45]. They produce chronic and intractable infection but with minimal inflammation.

Zoophilic Dermatophytes are fungal species inhabitating domestic and wild animals eg-Microsporum canis, Trichophyton equinum.Geophilic Dermatophytes are fungal species isolated from soil. They are less pathogenic eg-M.gypseum, M.nannum.The infection produced by geophilic and zoophilic species can produce severe inflammation.

3.2 EPIDEMIOLOGY

Generally Dermatophytes have universal distribution but some species are endemic to certain parts of the world. Trichophyton soudanense, Trichophyton gourvili, Trichophyton yaoundei are geographically restricted to Central and West Africa. Microsporum ferrugineum is predominantly seen in Japan and surrounding areas. Trichophyton concentricum, the etiological agent of imbricate is confined to South America[2]. Disruption of this epidemiological pattern occurs due to increasing mobility of world population[83]. The most common cause of tinea capitis in children in India, Canada, Nepal and Europe is Trichophyton tonsurans, but Trichophyton rubrum continues to be predominant in Tamil Nadu followed by Trichophyton mentagrophytes[59,60,78].

3.3 TAXONOMY OF DERMATOPHYTES

Kingdom	:	Eumycota
Phylum	:	Ascomycota
Class	:	Euascomy
Order	:	Onygenales
Family	:	Arthrodermataceae
Genus	:	Trichophyton 24 species
		Microsporum 16 species
		Epidemophyton 2 species

3.4 CLASSIFICATION OF DERMATOPHYTES

Dermatophytosis is classified based on their natural habitat into Ecological distribution, Morphological and clinical presentation.

TABLE 3.1 ECOLOGICAL CLASSIFICATIONS

	Anthropophilic	**Geophilic**	**Zoophilic**
Origin	People	Soil	Animals other than humans
Relative number of conidia in culture	Few	Most	Moderate
Human tissue response	Mild	Severe	Moderate
Examples	Epidermophyton floccosum	Microsporum gypseum	Microsporum canis Trichophyton equinum

TABLE 3.2 MORPHOLOGICAL CLASSIFICATIONS

	Epidermophyton	**Microsporum**	**Trichophyton**
Tissue attacked	Skin,nails	Hair,skin	Hair,skin,nails
Microconidia	None	Relatively few	Many
Macroconidia	Smooth thin walls, sparse in number	Thick, rough walls, many present	Smooth thin walls, relatively few present
Telomorph	Not yet discovered	Arthoderma(formerly Nannizia)	Arthoderma
Fluorescence in tissue	No	Characteristic of some species	No

3.4.1 CLINICAL CLASSIFICATION

Depend upon the anatomical site involved the clinical manifestation of dematophytes are named as follows[46]

- Tinea capitis- This is infection of shaft of the scalp hair. It may be inflammatory or non inflammatory[61]. The predominant causative fungal species of tinea capitis belongs to genus Trichophyton

- Tinea barbae – This is an infection of moustache and beard areas and is also called as barber's itch. Trichophyton verrucosum, Trichophyton mentagrophytes are the commonest causative organism

- Tinea faciei – Dermatophytic infection of the non bearded regions of the face.

- Tinea corporis – Dermatophytic infection of the glabarous skin of the body[73].

- Tinea cruris – Dermatophytic infection of groin and involves perineum, scrotum and perianal area and spread to inner third of the buttocks and occasional to thighs. It can be seen in inter triginous area such as under pendulous breast, axilla and around umbilicus of obese patients. It is also

called as jock itch, as itching is the predominant feature limited to inguinal area[38,39]. Trichophyton rubrum and Epidermophyton floccosum are the common causative agents[41].

- Tinea mannum - Dermatophytic infection of the palmar aspects of the hands. Mostly caused by anthrophilic species like Trichophyton rubrm, Trichophyton mentagrophytes and Epidermophyton floccosum

- Tinea pedis – Infection of the plantar aspect of the foot, toes and inter digital web space, popularly known as athlete's foot.

- Tinea unguim – It is the dermatophytic infection of the nail plates

- Tinea gladiatorum- Emerging infection in wrestlers involving arms, trunk, head and neck. Trichophyton tonsurans is the commonest isolate[90].

- Tinea imbricata – Unusual form of tinea corporis caused by Tinea concentricum [39,40]

- Tinea incognito – Clinical appearance of this dermatophyte infection has been modified by prior application of corticosteroids, nonsteroid topical immunomodulator like tacrolimus and pimecrolimus. Typical clinical

appearance is modified due to disturbance in classical clinical pattern of particular Tinea leading to delaying diagnosis.

- Tinea favosa – It is a chronic severe infection of the hair follicles with pus formation. It produces a cup shaped scutula. Scarring alopecia can occur in chronic favus infection. Usually caused by T.schoenleinii.

TABLE 3.3 CLINICAL CLASSIFICATIONS

Clinical types	**Involved anatomical site**	**Causative Dermatophytes**
Tinea capitis	Infection of shaft of the scalp hair	Trichophyton spp., Microsporum spp.,
Tinea barbae	Infection of beard and moustache areas of face	T.verrucosum, T.mentagrophytes
Tinea corporis	Glabrous skin of body	T.rubrum
Tinea imbricata	All hairless skin, sometimes nails, palms and soles.	T.concentricum
Tinea cruris	Groin area	E.floccosum, T.rubrum, T. mentagrophytes
Tinea unguium	Nail plates	T.rubrum, T.mentagrophytes, E.floccosum
Tinea manuum	Skin of palmer aspect of hands	T.rubrum,E.floccosumT.mentagrophytes
Tinea pedis	Plantar aspect of foot, toes, interdigital web spaces	T.rubrum, T.mentagrophytes, E.floccosum

The morphology and cultural characters of the most common dermatophytes are as follows.

3.5 TRICHOPHYTON SPECIES

This species usually infect skin, hair and nail. Macroscopically the colonies of Trichophyton species are powdery, velvety and waxy with characteristic pigmentation.Microscopically, both types of micro and macro conidia are seen but microconidia are the predominant spores which are arranged singly or in clusters along the hyaline septate hyphae.The macroconidia are sparse, smooth walled, pencil shaped with blunt ends and with 1-12 septations.The different species are Trichophyton rubrum, Trichophyton rubrum downy strain, Trichophyton rubrum granular strain, Trichophyton mentagrophytes var. mentagrophytes, Trichophyton mentagrophytes var. quinckeanum, Trichophyton tonsurans, Trichophyton schoenleinii,Trichophyton soudanens,Trichophyton verrucosum, Trichophyton violaceum Trichophyton ajelloi, Trichophyton concentricum, Trichophyton equinum.

3.5.1 TRICHOPHYTON RUBRUM

Trichophyton rubrum is the commonest species isolated from human[3,42,43]. It is an anthropophilic fungus. It may be of two types Macroscopically the downy type shows white downy to fluffy colonies on Emmons modified Neutral sabouraud dextrose agar after 10-14 days of incubation at 25° - 30°C.The reverse pigmentation is yellow to blood red[25]. Microscopically, the downy type produces moderate numbers of tear-drop microconidia which are arranged in bird on fence arrangement and no macroconidia[11,18]. Macroscopically, the granular type shows rugose fold with granular texture due to the production of macroconidia.The reverse pigmentation may be colourless, tan or yellow to brown; eventually a deep wine red colour appears. Microscopically it produces thin-walled; pencil shaped numerous macroconidia with 3-8 septations. They are borne directly on the hyphae with broad bases of attachment. Hair perforation test and urease test are negative.

3.5.2 TRICHOPHYTON MENTAGROPHYTES

This species is also common in human and animals.It is both anthropophilic and zoophilc.It has been associated with Tinea mannum, , Tinea corporis, Tinea unguium, Tinea pedis, Tinea capitis, Tinea cruris and Tinea barbae.Trichophyton mentagrophytes var . Mentagrophyes causes acute but severe inflammatory reaction.Trichophyton mentagrophytes var. interdigitale causes more chronic but less inflammatory reaction. After 6-8 days on modified Sabouraud's dextrose agar,at 25°-30°C,it produces flat,granular creamy colonies with reddish brown reverse pigmentation.Microscopically, thin,smooth walled,cigar shaped,3-6 celled macroconidia are borne on a branched hyaline septate hyphae by a narrow pedicle(rat tail conidia). Microconidia are globose and unicellular, produced either singly along the hyphae on short pedicle or en grappe.Trichophyton mentagrophytes var. interdigitale produces flat and downy colonies with white feathery fringes that may become pink with yellow to yellowish orange reverse pigmentation.Spiral hyphae, racket hyphae,favic chandeliers and nodular bodies are seen.Ectothrix hair do not fluoresce under wood's lamp.Hair perforation and urease test are positive.

3.5.3 TRICHOPHYTON SCHOENLEINII

This anthropophilic fungus sporadically forms Favus type of Tinea capitis with mouse like odour and scutula formation around the infected hair follicle, which result in permanent hair loss if not treated promptly. Endothrix pattern of hair produces pale greenish yellow fluorescence under Wood's ultra-violet light. Favus has been reported in Africa, Europe and Asia. After 2-3 weeks incubation on modified Sabouraud's dextrose agar at 25°-30°C produces waxy, heaped or folded with glabrous texture initially, became velvety on prolonged incubation. No reverse pigmentation is present. Difficult to maintain their cultures in typical convoluted form which rapidly become flat and downy. Microscopically, branched septate hyaline sterile hyphae are seen. Neither macroconidia nor microconidia are produced in routine cultures. But characteristic "favic chandeliers" are observed. On polished rice grain medium some isolates produces distorted clavate microconidia. Hair perforation test is negative.

3.5.4 TRICHOPHYTON TONSURANS

This anthropophilic fungus produces Black dot Tinea capitis in children which involves scattered hairs with little inflammation or itching. A fine red scaly lesions of skin and occasionally Tinea unguium and Tinea pedis can occur. Do not fluorescence under Wood's ultra-violet light. After 7-14 days incubation at 25°-30°C on modified Sabouraud's dextrose agar, it produces obverse yellow colonies with a powdery texture with radial grooves and reverse yellow-brown to reddish-brown pigmentation. Hyphae are hyaline septate and branched with terminal swelling, and barrel shaped arthroconidia are seen in chains.Short and blunt irregularly clavate or cylindrical shaped 3-8 celled macroconidia are sparsely seen. Numerous characteristic ballooning of microconidia with various shapes and size are borne at right angles to the hyphae[65,68,90].Intercalary chlamydoconidia, racquet hyphae and spiral hyphae are also seen. Hydrolysis of urea is positive at 5 days. Hair perforation test is positive within 14 days.

3.5.5 TRICHOPHYTON VERRUCOSUM

This zoophilic fungus causing highly inflammatory dermatophytoses in cattle and humans which are worldwide in distribution. Suppurative Tinea corporis, kerions, Tinea barbae and Tinea mannum and ectothrix type of Tinea capitis which fluoresce under Wood's ultra-violet light are also common.

On modified Sabouraud's dextrose agar supplemented with thiamine and inositol, at 25°-30°C, after 3-4 weeks of incubation, colonies are white, slightly folded with glaborous texture. Typical colonies are deeply submerged in the medium. Reverse of the colonies are usually colourless or salmon coloured. Microscopically, distorted hyaline septate hyphae with intercalary chlamydoconidia produced at 37°C and favic chandeliers are seen.Few strains have shown to produce microconidia ranging from clavate to pyriform forms which occur singly along their corresponding hyphae on thiamine media .In the same instance, macroconidia if ever occurs has a characteristic rat tail appearance.

3.5.6 TRICHOPHYTON VIOLACEUM

Considered to produce a long standing non- inflammatory type of lesion described as black dot, trichophyton violaceum has a geographic distribution all over the world. Producing lesions which when examined under woood's ultraviolet light show no fluorescence, this fungus has an endothrix formation on the affected hairs. Permanent alopecia and kerions can occur.On modified Sabouraud's dextrose agar, after 14-21 days, at 25°-30°C, cream coloured, glaborous, heaped ,lavender coloured colonies are produced. This fungus is notorious to grow in a pleomorphic fashion which when examined appear as white sectors and nonpigmented strains also occur on few instances[34,35].The reverse pigment of culture is lavender to purple. Microscopically, sterile distorted, twisted, branched, septate hyaline hyphae, chains of intercalary and terminal chlamydioconidia, swollen hyphal cells containing cytoplasmic granules and favic chandeliers are seen. This fungus has never shown to produce conidia, but few reported instances of pyriform conidia have shown to develop on media enriched with substances.Hair perforation test is negative[94].

3.6 MICROSPORUM SPECIES

Microsporum species infect skin and hair but not nails.The colonies are cottony with white to brown pigmentation. Multiseptate (1-15), fusiform, spindle-shaped, echinulate macroconidia 6 - 160 × 6 - 125 μm in size, are formed at the ends of the hyphae. Pyriform to clavate shaped single-celled, smooth-walled microconidia may be produced in some species.

3.6.1 MICROSORUM GYPSEUM

This geophilic fungus causes Tinea capitis and Tinea corporis infection. It has geographical distribution of all over the world[50].Solitary raised boggy skin lesion and ectothrix type of hair infection which fluoresce dull yellow-green under Wood's ultra-violet light are produced [21,58].

After 5-6 days of incubation at 25°C, the culture shows white and downy colonies later becomes flat and granular with fringy edges.The reverse pigment is tan to orange brown or modified cinnamon pink. Microscopically, hyaline, septate, branched hyphae with echinulate 2-6 celled ellipsoidal

macroconidia are seen in clusters. The macroconidia has rounded distal and truncated proximal ends. Ragged annular fringes of macroconidia are seen.Hair perforation test is positive.

3.6.2 MICROSPORUM CANIS

This zoophilic dermatophyte is the common cause of ringworm in children because certain fungicidal fatty acids are absent in them and frequent contact with cats and dogs. Tinea corporis and acute inflammatory ectothrix pattern of grey patch Tinea capitis occurs. Greenish-yellow fluorescence is produced in Wood's lamp.After 4-6 days incubation at 25°C, produces white cottony colonies with radial grooves in Emmons neutral sabouraud‘s dextrose agar. The reverse pigment is yellow orange to brownish yellow reverse pigment.Microscopically, hyaline, septate, branched, hyphae with abundant spindle shaped, echinulate, 6-15 celled, asymmetrically beaked apex macroconidia are abundant and pyriform shaped microconidia are sparse. Pectinate bodies, nodular bodies, racquet hyphae and chlamydoconidia are present. Hair Perforation Test is postive at 14 days.

3.6.3 MICROSPORUM NANUM

It is contracted from contaminated soils and infected pigs. It produces Tinea corporis and ectothrix pattern of Tinea capitis, will not produce any fluorescence in Wood's lamp.

On modified Sabourauds dextrose agar, after 7 days, at 25°C, a colony are initially flat, powdery white and downy, later becomes cream to deep tan with reddish tint and brownish red reverse. Microscopically, the hyaline, septate, branched, hyphae bears 2 celled echinulate, ellipsoidal macroconidia and sessile, smooth walled, clavate to pyriform microconida.Hair perforation test is positive

3.7 EPIDERMOPHYTON SPECIES

It affects mainly the skin and nails but not the hair. The genus of Epidermophyton has only one species.The colonies are slow growing, powdery and khaki coloured which do not produce microconidia. Smooth, thin-walled, 1-9 celled pear shaped macroconidia are arranged in clusters.

3.7.1 EPIDERMOPHYTON FLOCCOSUM

This anthropophilic dermatophyte has a wide geographical distribution and cause skin and nail infection. Epidermophyton floccosum is sensitive to cold temperature, so the specimens should not be refrigerated.After 6-12 days of incubation in modified SDA at 25°-30°C, white and downy colonies, becomes velvety or powdery with yellowish-brown reverse pigment depending upon the macroconidia are produced. The obverse colour is mustard yellow or khaki with yellow fringe.

Microscopic morphology shows thin, hyaline, septate, branched hyphae, macroconidia are smooth, thin –walled, 5 celled, beaver's tail appearance arranged in clusters. Many chlamydoconidia, few spiral hyphae, racquet hyphae and nodular bodies are seen. No microconidia are formed.

3.4 DISTRIBUTION OF CONIDIA OF DERMATOPHYTES

Dermatophytes	**Macroconidia**	**Microconidia**
Trichophyton	Rare, thin walled smooth	Abundant
Microsporum	Abundant,thick walled, rough	Rare
Epidermophyton	Abundant , smooth walled	Absent

3.8 PATHOGENESIS AND PATHOLOGY

3.8.1 SOURCE OF INFECTION

Infection may be acquired from the transfer of arthroconidia or hyphae, or keratinous material containing these fungal elements from an infected host to a susceptible uninfected host.

Dermatophytes grow only within dead keratinized tissue. Keratinolytic proteases produced by fungal cells help the fungal metabolic products to enter into the living cells by diffusion through malphigian layer of the epidermis[17,20]. It causes pruritis and erythematous annular lesion with central clearing.

3.8.2 MODE OF TRANSMISSION

Through infected person / animals and auto inoculation are from another body site. Transmission of dermatophytes occur either through human-human (anthropophilic), animal-human (zoophilic) or soil-human/animal (geophilic). It occurs more often in the tropics and seen predominantly in men due to their increased activity and sweating. Aggravating factors include moisture; occlusion and trauma.The chronicity of T. rubrum infection is due to the presence of mannans in the cell wall of dermatophytes by immuno-inhibitory effects. The invasion of dermatophytes depends on the host factors including protease inhibitors and hormones.

The factors that limit the infection to the keratinized tissue includes the preference of dermatophytes to cooler temperature of skin compared to the normal body temperature, serum factors like ß globulins, ferritin other metal chelators. The host immunological factors determine the clinical course of the disease.

Dermatophytid or Id reaction is a secondary eruption occurring in sensitized Tinea patients because of the circulation of allergenic products from primary site of infection. It is an itchy, painful lesion that does not contain the causative fungi, which occurs in patients with absence of delayed reaction to dermatophytic antigen(Trichophytin) or following commencement of oral antifungal therapy due to the absorption of fungal products from skin. Two main types of id reactions are

- Lichen scrofulosorum – like
- Pompholyx – like
- Lichen scrofulosorum-like

It is associated with Tinea capitis especially kerions in children. The follicular lesions are symmetrical and centrally distributed that are diffusely scattered with horny spines at the top of the follicles.

- Pompolyx-like

This is a type three hypersensitivity reaction with lesions on the sides and flexor aspect of the fingers and palms in patients with Tinea pedis. The dermatophytic reaction responds to desensitization and resolves spontaneously with treatment of primary disease.

3.8.3 IMMUNOLOGY

The inflammatory host response due to fungal exoenzyme plays a major role in the pathogenesis of dermatophytosis. Variety of antibodies is produced by host due to dermatophytic infection. IgE aids in suppression of cell mediated immunity by modulating histamine activity. The humoral immunity response had a varied presentation due to lack of standardized antigens. But cell mediated immunity is the corner stone of host defence in dermatophytosis.

Cell mediated immunity is the main defence mechanism expressed by delayed type hypersensitivity response to dermatophyte antigen (trichophytin).Cell mediated immunity is responsible for spontaneous healing in case of acute infection but plays no role in case of chronic infections. Immune response to dermatophytic infection has been demonstrated in both human and animals such as calves, rabbits, guinea pigs, and rats. Live vaccine (LTF130) against Trichophyton veruccosum are used in some countries like Soviet Union and Eastern Europe, but it is not effective in humans.

3.9 CLINICAL FEATURES

Depending upon the anatomical site involved tinea or ring worm infections are classified. The second part of the name of the dermatophytosis identifies the part of the body infected. Tinea is a Latin word meaning 'worm' or 'moth' because of the serpentine (snake like) and circular or annular (ring like) lesions that occur in skin. The literal meaning of the word 'dermatophyte' is 'skin plant' which is a misnomer as the fungi are phytogenitically not related to plants.

The clinical manifestation depends upon the species of the fungus, inoculum size, and involved body site and host immune condition[63]. The common clinical conditions produced by the dermatophytes are Tinea Corporis, Tinea cruris, Tinea mannum, Tinea barbae, Tinea capitis, Tinea pedis and Tinea unguium[72].

3.9.1 TINEA CORPORIS

It is the dematophytic infection of the glabrous skin of the trunk and extremities .The disease is characterized by erythematous scaly lesion, any dermatophyte can potentially cause Tinea corporis, but T.mentagrophytes is one of the common pathogen only next to T.rubrum. Domestic animals are the important factors in transmission of organisms causing Tinea corporis specifically the zoophilic types. Important risk factors in acquiring Tineacorporis are having personal history of or close contact with Tinea capitis or Tinea pedis. Other predisposing factors include occupational or recreational exposure (military housing, gymnasiums, locker rooms, outdoor occupations, wrestling), contact with contaminated clothings and furniture and immunosuppression.Its incubation period is 1 to 3 weeks. Lesions may be arcuate, circinate, oval in shape, scaly (lessened or absent in corticosteroid use-tinea incognito).

Lesions may be vesicular, granulomatous or verrucous in appearance. Associated symptoms include pruritis and burning. Clinical variants of Tinea corporis include Tinea profunda, Majjochi's granuloma and Tinea imbricate. Tinea profunda results from excessive inflammatory response to a dermatophyte (analogous to a kerion on the scalp). Majjoci's granuloma caused by T.rubrum is characterized by perifollicular pustules or granulomas. T.imbricata caused by T.concentricum is a chronic infection presenting as concentric annular rings[73].

3.9.2 TINEA CRURIS

Is the dermatophyte infection of the inguinal region particularly inner aspect of upper thigh and crural folds. Three most common causative agents as E. floccosum, T.rubrum and T.mentagrophytes. mentagrophytes. Men more commonly affected-scrotum encourages a warm and moist environment. Other predisposing factors are obesity and excessive perspiration. Initial sign is an area of erythema and pruritis in the intertrigenous fold between the scrotum and thigh. Followed by sharply demarcated with a raised erythematous scaly advancing border, border may contain pustules or vesicles.

3.9.3 TINEA MANNUM:

Is the infection of the palm and the inter digital space thought to be related to the lack of sebaceous glands in the palm. Causative organisms- E.floccosum, T.rubrum and T.mentagrophytes.Usually associated with Moccasin type Tinea pedis.

3.9.4 TINEA BARBAE

Dermatophytosis limited to postpubertal males and involves the bearded areas of face and neck. Causative organism is T.mentagrophytes var mentagrophytes and T.verrucosum. Others are T.schonleinii, T.violaceum and T.megninii. Common cause of infection was contaminated razors in barber shops. Because zoophilic organisms are the common cause and due to the large amount of terminal hair follicles in the affected areas the clinical presentation tends to be severe with intense inflammation and multiple follicular pustules .Abcesses , sinus tract, bacterial super infection and even kerion like lesion may develop.

3.9.5 TINEA CAPITIS:

Children are most commonly affected. Causative pathogens are T. tonsurans, M.canis and M. audouinii.Three pattern of invasion exists. Endothrix pattern results from infection with anthropophilic fungi in the genus Trichophyton and is characterized by nonfluorescent arthroconidia within the hairshaft. The clinical presentation varies from scaling to black dots with patchy alopecia to kerion formation. T.tonsurans and T.violaceum are important causes of endothrix formation[81,84].

Ectothrix pattern occurs when arthroconidia are formed from fragmented hyphae outside the hairshaft. Ectothrix infection may be fluorescent or nonfluorescent as determined by wood's lamp examination.

T.schoenleinii is responsible for Favus, a severe form of dermatophyte hair infection with thick yellow crusts and white fluorescence under woods lamp examination . Scarring alopecia may develop in chronic cases.

3.9.6 TINEA PEDIS

The dermatophytic infection of the soles and interdigital web spaces. Most common infection around the world affecting both sexes.The lack of sebaceous glands and the moist environment caused by occlusive shoes are the important factors in development of tinea pedis. Most believe that it is acquired by walking bare foot. No specific susceptibility has been determined to explain why some people are more likely to than others to acquire the disease despite the same level of exposure. The common pathogens are T.rubrum, T.mentagrophytes, E.floccosum and T.tonsurans. Based on the clinical presentation it may be divided into four types as Interdigital, Hyperkeratotic, Ulcerative and Vesicular.

3.9.7 TINEA UNGUIUM (ONYCHOMYCOSIS)

Onychomycosis comprises of all fungal infections affecting the nail apparatus, i.e., nail matrix, nail plate, cuticle, mesenchymal tissue and nail folds[62]. In spite of improved personal hygiene and living environment onychomycosis continues to spread and persist. The prevalence rate of onychomycosis is determined by age, predisposing factor, social class,

occupation, climate, living environment and frequency of travel. It is divided in to three patterns based on the point of fungal entry into the nail unit.

- Distal / lateral subungual with invasion via the hyponychium (most common)
- White superficial with direct invasion into the superficial nail plate (often due to T.mentagrophytes)
- Proximal subungual with direct invasion under the proximal nail fold (immunocompromised host)

The common pathogens are T.rubrum, T.mentagrophytes and E.floccosum (less frequently Microsporum spp). As single nail may be involved but more commonly multiple nails on one or both hands or feet affected. The responsible organism initially invades the nail bed in the region of hyponychium leading to hyperkeratosis of the nail bed. With further progression on infection there is yellowing and thickening of the distal nail plate as well as onycholysis which is an ideal environment for further proximal invasion and growth of the dermatophyte. Eventually the entire nail bed and plate may become involved (total dystrophic pattern). Serious complication such as cellulitis may arise from onychomycosis especially in patients who are diabetic or immunocompromised[84,86,87].

3.10 LABORATORY DIAGNOSIS

3.10.1 SPECIMEN COLLECTION

After decontaminate the affected area with 70% alcohol and allow it for air dry to remove as much of the normal skin flora as possible and to obtain tissue from area to contain viable organism. The lesion was scrapped at its active edge by blunt scalpel

3.10.2 TRANSPORT OF SAMPLES

The samples were collected and transported in the dark papers. The important factor is to keep the specimen dry. It prevents the bacterial contamination. Containers such as test tubes that allow moisture to condense around the specimen should not be used. In the laboratory, diagnosis depends on the demonstration of causative pathogen in tissue by microscopy, isolation of fungus in culture and the serological tests.

3.10.3 WOOD'S LAMP EXAMINATION

Wood's lamp is a device that is useful in the diagnosis and management of superficial cutaneous fungal infections. Its an important the advancement in medical mycology. Woods glass was made up of barium silicate which contains about 9% nickel oxide. It transmits 365nm ultra violet light that shows characteristic fluorescence by some microbial agents, so survey of large population can be done rapidly and easily. The chemical substance responsible for positive fluorescence is pteridine. The false positive results are due to ointments containing petroleum jelly, serum exudates, lint and dried soap. Its use is limited nowadays because of the increase in prevalence of non fluorescing fungi like Trichophyton tonsurans and Trichophyton verrucosum.

Micro organisms		**Fluorescence Colour**
Microsporum canis	:	Bright green
Microsporum audouinii	:	Bright green
Microsporum ferrugineum	:	Blue green
Microsporum distortum	:	Blue green
Microsporum gypseum	:	Dull yellow
Trichophyton schoenleinii	:	Dull green

3.10.4 DIRECT MICROSCOPIC EXAMINATION

Clinical materials like skin scales, nail clippings and hair stubs are usually examined under direct microscopy is the most rapid ,simple and effective screening methods of detecting the etiological agent of fungal infection[76,77].

Nail clippings stained with periodic acid-Schiff stain or Methenamine silver stains are more rewarding as compared to 10 %KOH wet mount.

3.10.4.1 POTASSIUM HYDROXIDE MOUNT

A clean dry glass slide is taken and collected samples are mounted with 10-20% KOH, then covers with a cover slip and gently heated in a Bunsen flame to facilitate keratinolysis[19]. It aids the highly refractile branched hyaline septate hyphae visible clearly. In hair both ectothrix and endothrix pattern were identified by appreciating the presence of arthroconidia.

➢ **MODIFICATION OF KOH**

- ❖ By using superchrome parker blue-black ink along with KOH solution.
- ❖ When 36% Dimethyl sulphoxide(DMSO) was used along with
- ❖ 20% KOH helps in better penetration of the stain into the tissues without heating.
- ❖ To prevent dehydration and crystallization of KOH, 5-10 percent of glycerine is used.

The mounted KOH slide was examined under microscope with both low power and high power objective lens. But it needs some experience to differentiate the fungal hyphae from other artefacts like cotton wool, synthetic fibres and cholesterol crystals.

3.10.5 CALCOFLUOR WHITE

This fluorescent dye is used in textile industry as whitening agent .It combines with the chitin layer of the fungal cell wall and produces fluorescence under ultraviolet rays, but it requires expensive special fluorescent microscope with appropriate ultraviolet source and 300-412mm radiation filters [47,49].

3.10.6 PERIODIC ACID-SCHIFF (PAS) STAIN

It reacts with the fungal cell wall polysaccharide and produce red violet fuchsin colour in the tissues and is used for histopathological examination of the nail samples. Fungal infection cannot be ruled out by this presumptive diagnostic method

3.10.7 FUNGAL CULTURE

Fungal culture is a confirmatory test used to identify the causative fungal agent and helps to select the specific antifungal therapy Dermatophytes can grow easily on Emmon's modified sabouraud dextrose agar with pH close to neutral (6.8/7)by reducing dextrose content from 40 to 20g/L to which add antibiotics such as Gentamicin 20mg, Chloramphenicol 50 mg and cycloheximide 500 mg to the boiling media. Mix it properly and dispense it in tubes and autoclave at 121°C for 15 minutes. The clinical specimen should be inoculated on fungal culture medium in duplicate, irrespective of the direct examination findings and incubated at 25° C, 30° C and 37 ° C. The dermatophyte isolate can be identified by the colour and compact growth around inoculum within 10 days to 3 weeks time. The three genera of

dermatophytes are recognized based on morphology of macroconidia and microconidia, their shape and position on spore bearing hyphae.The associated features such as spiral hyphae, racquet hypha, nodular organ, pectinate body and favic chandelier also helps for the identification of different species of dermatophytes.

3.10.7.1 RIDDLE'S AGAR BLOCK METHOD

The slide culture is used to study undisturbed morphological details particularly relationship between reproductive structure like conidia, conidiophores and hyphae. Fungal slide culture was performed in cases with doubtful morphology [23,49].

Placed a sterile microscope slide on a bent glass rod at the bottom of a Petri dish. A piece of one square centimeter block of sabouraud dextrose agar or potato dextrose sugar was put up on the slide. Inoculate the fungal strain under identification at four sides of agar block. Then place a sterile coverslip over it and incubated at 25°C in BOD incubator. Add a little water on the filter paper to avoid drying of agar.

After full growth, the cover slip is lifted vertically from the agar block and mounted over the lactophenol cotton blue stain and examined under

microscope to identify the fungus. The mycelia which adhere to the glass surface usually show characteristic microscope appearance which may be lost if teasing needle are used as happens in the routine LPCB mounts. The slide culture was also directly examined by putting under low power of microscope.

The cellophane tape preparation has come into greater use to overcome the obstacles of time consumption and requirement of the extra equipment to prepare the slide culture. A piece of tape is gently laid over a portion of the fungal colony and slowly lifted to remove an area of the LCB on a microscopic slide and covered with cover slip. This preparation becomes an instant slide culture, revealing relationship of the various fungal structures.

3.10.8 DERMATOPHYTE TEST MEDIUM

Dermatophytes test medium is available commercially, contains cycloheximide to inhibit saprophytic fungi. It also contains choloramphenicol to suppress the bacteria growth. The growth of the dermatophytes can be detected by the change in colour due to the metabolic activity of the fungi and makes the medium alkaline by shifting the pH which was detected by the phenol red indicator some non pathogenic fungi can also produce similar colour change like the dermatophytes in this medium. It is used to ioslate and

distinguish dermatophytes from the fungal or bacterial contaminants found in cutaneous lesions[95]. They turn the medium red by raising the pH through metabolic activity while most fungi and bacteria do not.

Specializied mediums like casaminos erythritol albumin medium, bromocresol purple casein yeast extract agar and 0.1 percent yeast extract or thiamine containing medium may also be used in the isolation of dermatophytes, particularly T.verrucosum.

3.10.9 PHYSIOLOGICAL TESTS

Along with gross and microscopic examination some physiological tests are also used for the identification of the species.

3.10.9.1 IN VITRO HAIR PERFORATION TEST

Hair Perforation Test is performed to differentiate between Trichophyton mentagrophytes and Trichophyton rubrum as well as Microsporum canis and Microsporum equinum, respectively. The test is taken as positive when the dermatophyte species show wedge-shaped perforations in the hair. It is

positive in Trichophyton mentagrophytes and Microsporum canis and negative in Trichophyton rubrum and Microsporum equinum. This test is also helpful in identifying other dermatophyte species.

3.10.9.2 UREASE TEST

Christensen's urease agar or broths medium are used to differentiate Trichophyton rubrum and Trichophyton mentagrophytes. Trichophyton mentagrophytes hydrolyse urea and the medium becomes deep red while Trichophyton rubrum shows negative result.The dermatophyte Trichophyton raubitschekii a variant of Tichophyton rubrum is urease positive. Trichophyton megninii is positive in a urea-indole broth but negative in Christensens's urease medium. If no colour change occurs within 7 days of inoculation at 23°C-30°C, the test is declared negative.

3.10.10 NUTRITIONAL REQUIREMENTS MEDIUM

3.10.10.1 TRICHOPHYTON AGAR

This is commercially available medium contains vitamins and amino acid. Based on their requirements of the special growth factors, this medium is used to differentiate tichophyton species.

Trichophyton agar No 1: It is a casein basal medium contains no vitamins.

Trichophyton agar No 2: It contains Inositol.

Trichophyton agar No 3: It contains Thiamine and Inositol.

Trichophyton agar No 4: It contains Thiamine.

Trichophyton agar No 5: It contains Nicotinic acid.

Trichophyton agar No 6: It contains Ammonium Nitrate.

Trichophyton agar No 7: It contains Ammonium Nitrate and histidine.

3.10.11 ADDITIONAL TESTS

Bromocresol milk solids glucose agar, auto claved polished rice grains and test for temperature tolerance are used for identification of dermatophytes [2,26].

3.10.12 IMMUNODIAGNOSIS

A delayed type hypersensitivity skin tests with dermatophytic antigen, trichophytin and serological tests are important for the diagonosis of the dermatophytosis[52,53]. The reactive component of the antigen is the galactomannan peptide. Immediate response is due to the carbohydrate portion whereas peptide moiety is associated with immunity.

Various serological test like immunodiffusion are done to establish the diagnosis of dermatophytosis[22,27]

3.10.13 MOLECULAR ANALYSIS

Clinically significant dermatophytic species are identified by utilizing the Single primer simple repetitive oligonucleotide(GACA) in the PCR fingerprinting Chitim synthase 1 gene analysis of dermatophytes has also been utilized[36].

3.10.14 ANIMAL PATHOGENICITY

This is useful for the laboratory study of nature of lesions and immunity produced by the organism. Animal pathogenicity Testing is done on the guinea pigs. Microsporum canis, M.gypseum and T.mentagrophytes may be established more readily in the laboratory animals as compared to other species. The hairs of the area to be infected (usually the dorsal part) are shaved and the skin is scarified before applying the conidial and hyphal suspensions[65,76]. The lesions develop within a week and resolve after three to four weeks in most of the cases.

3.10.15 ANTIFUNGAL AGENTS USED IN DERMATOPHYTOSIS

ANTIFUNGAL DRUG CLASSIFICATION

- **Antibiotics**
  - Polyenes:

    Amphotericin B, Nystatin,Hamycin, Natamycin
  - Heterocyclic benzofuran

    Griseofulvin
- **Antimetabolite:**
  - Flucytosine(5-FC)
- **Azoles**
  - Imidazoles
    - Topical

      Clotrimazole, Econazole, Miconazole, Oxiconzole
    - Systemic

      Ketaconazole

- **Trizoles**

  Fluconazole, Itraconazole, Voriconazole

- **Allylamine**

  Terbinafine

- **Other topical Agetns**

  Tolnaftate, Undecylenic acid, Benzoic acid,Quiniodochlor, Ciclopirox olamine

3.10.15.1 ANTIFUNGAL ANTIBIOTICS

Griseofulvin –It inhibits fungal mitosis by interference with polymerized microtubule and spindle formation in diving cells. It is a fungi static drug and very effective in all forms of dermatophytosis.

3.10.15.2 SYNTHETIC ANTIFUNGAL AGENTS

Thiocarbomate-Tolnaftate, it can be used simultaneously with systemic therapy in Tinae corporis and Tinea cruris. Allylamines-These agents selectively inhibits the key enzyme squaleneepoxidase which is required for fungal ergosterol biosynthesis and leads to accumulation of squalene which leads to weakening of cell membrance and cell death[55,64]. The following allylamines are clinically significant Naftifine, Naftifine and Terbinafine. Terbinafine has high potency against dermatophytes as shown by its very low MIC values[82,102].

- **Benzylamines**

Butinafine is a new benzlamine derivative with clinical structure and mode of action similar to allylamine antifungal agents.

- **Azoles**

The principal mode of action of the azole is impairing the ergosterol synthesis by inhibiting cytochrome P-450 enzyme which results in accumulation of abnormal sterols and production of defective cell wall and

ultimately leads to fungal death. The common drugs are Imidazoles, Miconazole,Clotrimazole, Ketoconazole and Econazole.

Triazoles-Fluconazole has poor water solubility, oral absorbtion, extensive bio-availability independent of food or gastric pH least protein binding has sufficiently long half life to allow once a day administration, on preliminary clinical studies have reporded it to be effective against dermatophytes.

- **Miscellaneous antifungal agents**

  Cyclopiroxolamine

  Undecyclonic acid

  Whitfield's ointment

3.11 INVITRO ANTI FUNGAL SUSCEPTIBILITY TEST FOR DERMATOPHYTES

The National Committee for Clinical Laboratory Standards (NCCLS) M-38-A which describes the standard parameters for testing MIC of established agents against filamentous fungi. This has been modified with incubating temperature (28 versus 35^{o}C) and duration of incubation (4 to 10 days versus 21 to 72 hours) for testing dermatophytes M-38-P[67]. Antifungal susceptibility testing is receiving increased attention with the advent of newer anti fungal drugs. However susceptility testing of filamentous fungi is not as advanced as susceptibility testing of yeast. But it helps to monitor the development of resistance and predict the therapeutic potentials of newer drugs[51,70,71].

In vitro susceptibility testing of fungi depends upon many factors such as inoculum size, composition of the medium, pH, incubation duration, and temperature and MIC end point determination[24,16]. In addition, because of the slow growing nature of the fungi and their capacity of some of them to grow either as yeast with blastoconidia or as moulds with a variety of conidia

depend upon these conditions. The trailing end point observed with the azoles is another major problem encountered in the susceptibility testing of the fungi.

Several studies have attempted to correlate the MIC results with outcome. However only little evidence is available .The retrospective nature of the studies, the documented variability of the non-standardized in vitro methods and the difficulty in defining mycoses and their responses to therapy are responsible for this status[67].

The methods that have been most frequently applied to antifungal susceptibility testing are-Disc diffusion method, Episilometer test, Agar dilution test, Micro and macro broth dilution test, spectrophotometric methods and flowcytometry.

3.11.1 AGAR DILTUION METHOD

1.8ml of molten nutrient agar was taken and allowed to cool to 50°C.Then 0.2 ml of drug dilutions from stock solutions was added in descending concentration to NA slopes.10µl of standardized inoculums was

added to all tubes except sterility control tube. Tubes incubated at 35°c for 7 days visualized macroscopically for any growth. Lowest concentration of the drug which permitted no macroscopically visible growth after 7 days is taken as MIC[96,99].

3.11.2 MICRO BROTH DILUTION METHOD

To prepare 5 ml volumes of antifungal agent pipette 4.9 ml volumes of RPMI 1640 medium into each of 10 sterile test tubes. Now, using a single pipette add 0.1 ml of DMSO alone to one 4.9 ml lot of medium (control medium), then 0.1 ml of lowest (3.13 microgram /ml) drug concentration in DMSO, then 0.1 ml of the 6.25 μg/ml, concentration and continue in sequence up the concentration series, each time adding 0.1 ml volumes to 4.9 ml medium. These volumes were adjusted according to the total No. of test required. Because there will be 1:2 dilution of the drug when combined with the inoculums, the working antifungal solution are 2 fold more concentrated than the final concentration (NCCLS)[96].

7-15 days old cultures grown on SDA at 25^{o}C was used. Mature colonies were covered with 10ml of sterile saline (0.85%). Growth scraped by sterile

Pasteur pipette. Heavy particles allowed to settle for 15-20 minutes at room temperature. Supernatant was mixed with a vortex for 15 seconds. Turbidity of supernatant was adjusted spectrophotometrically to 530nm 65-70% absorbance. Each suspension was diluted 1:50 in RPMI 1640.

Each well will be inoculated on the day of test with 0.1 ml of 2x inoculums suspension. This step will dilute the drug concentration, inoculums densities, and solvent used to the final desired test concentration.

The growth control well contains 0.1ml of the corresponding diluted inoculums suspension and 0.1ml of the drug diluents without anti fungal agents.

Test was performed in sterile microtitre plates and incubated at 28^{o}C without agitation for 4 to 7 days.

MATERIALS AND METHODS

The cross sectional study about dermatophytosis comprised of 217 patients attending the dermatology clinic and was carried out in department of microbiology in Government Kilpauk Medical College and Hospital, Chennai for a period of one year to isolate and speciate the dermatophytes and to determine its antifungal susceptibility pattern.

4.1 INCLUSION CRITERIA

- Both male and female cases with all age groups of clinically diagnosed dermatophytic infection.
- All clinically diagnosed and untreated dermatophytosis cases were included in this study.

4.2 EXCLUSION CRITERIA

- Non dermatophytic skin infection.
- Patients already started on antifungal treatment will be excluded

4.3 SPECIMENS COLLECTION

4.3.1 HISTORY ELLICITED FROM THE PATIENT

All relevant details like age, sex, duration of complaint, distribution of lesion and history of previous similar complaints and treatment history for Diabetes, Tuberculosis, Neoplasms, HIV and surgeries[56,57,92]. Detailed histoy of exposure to animals, known cases, pets at home or any other suspected sources were collected[1,13].

- **SKIN SPECIMEN**

The affected area was thoroughly swabbed with 70% alcohol to remove surface contaminants. The alcohol was allowed to dry by evaporation[12]. The active edge of the lesion was scrapped with a flame sterilized blunt scalpel held at an angle of 90^{o} to skin surface.

- **HAIR SPECIMEN**

Scrapings with blunt scalpel which includes hair stubs, contents of plugged follicles and scales and affected hair were also epilated and care was taken to collect the basal portion of the hair as fungus was usually found in this area[21].

- **NAIL SPECIMEN:**

The diseased nail was meticulously swabbed by using 70% alcohol. After which the nail samples are clipped from the free edge and should also include its full thickness from the base of the nail[13].

4.4 TRANSPORT OF SAMPLES

The samples were collected in folded squares of sterilized dark papers for transport to laboratory thereby permitting drying and reducing bacterial contamination.

4.5 PROCESSING OF SPECIMEN

4.5.1 DIRECT EXAMINATION

The microscopic examination of 10% KOH wet mount of keratinous material is very simple and reliable.After heating the mounted slide in Bunsen flame which helps to clear the materials within 5-20 minutes. But crystallization of slide material will occur on prolonged heating.

A small amount of clinical specimens were mounted in 10% KOH solution on a clean glass slide. The wet mount was examined under both low power and high power objectives of light microscope for presence of septate hyphae. The ring worm fungi was differentiated from epidermal cell outlines, elastic, cotton and vegetable fibers and artefacts such as intra cellular cholesterol.

4.5.2 FUNGAL CULTURE

All the samples collected were inoculated on to Emmons modified Sabourauds Dextose agar containing cycloheximide (0.05mg/ml) and chloramphenical(0.05mg/ml) in duplicate irrespective of the findings of direct

examination to detect the growth of dermatophytes in the clinical sample.It has pH close to neutral for increasing recovery of fungi and reducing dextrose content from 40-20 gram per litre and contains neopeptone instead of peptone with final pH of 6.8±7.0 so it is called neutral sabourauds agar. The slopes were incubated at 25°C and 37°C for 4 weeks and examined daily in the first week, and twice a week thereafter for any fungal growth. Slopes not showing growth for 4 weeks were considered negative for growth. Identification was done on the basis of colony characteristics as well as microscopic morphology in Lacto phenol cotton blue mount.

4.5.3 DERMATOPHYTE TEST MEDIUM:

DTM contains actidione to inhibit saprophytic fungi. It also contain gentamicin and choloramphenicol to inhibit bacteria. DTM is used for the rapid identification and differentiation of the dermatophytes from fungal or bacterial contaminants found prevalent in cutaneous lesions[28,29]. The dermatophytes turn the medium red at 25°c, due to increased PH through their metabolic activity and indicated by the indicator phenol red present in the medium. While most other fungi and bacteria do not.

4.5.4 HAIR PERFORATION TEST

Some of the dermatophytic species produce a special hyphae called perforating organs which is capable of creating a perforation in hair. This invitro test is done to differentiate between Trichophyton mentagrophytes and Trichophyton rubrum as well as Microsporum canis and Microsporum equinum. It shows positive test in Trichophyton mentagrophytes and Microsporum canis but shows negative test in Trichophyton rubrum and Microsporum equinum.

- **PROCEDURE**

- By using test organism, lawn culture was made in potato dextrose agar plate.
- Prepubescent blonde child sterile hairs were placed, onto the fungal lawn.
- To accelerate the reaction, 5-6 drops of 1% yeast extract were added.
- It was incubated at 30°C for upto 28days and examined.
- Some amount of the hairs were taken and placed in a clean glass slide and add a drop of lactophenol- cotton blue ,then place a sterile coverslip over it.

- The test was considered positive when cone-shaped perforations was appreciated along the long axis of the hair[49].

4.5.5 UREASE TEST

Christensen's urease agar slant is used to diffentiate T.mentagrophytes from T.rubrum. T.mentagrophytes strain, hydrolyse urea and produces bright pink colour indicating the ph shift by the formation of ammonia. Urea broth may also be used which is more sensitive.

➢ PROCEDURE

- The urease agar slant was inoculated with test organism and incubate at 30°C for 7 days.
- The production of bright pink colour indicates positive reaction .

4.6 ANTIFUNGAL SUSCEPTIBILITY TESTING FOR DERMATOPHYTES

- **MICROBROTH DILUTION METHOD:**

REQUIREMETS:

Sterile test tubes for drug dilution/ inoculums preparation, sterile disposable microtitre plates, Micro pipette/ Sterile tips/ Gloves/ Disposable face masks.

MEDIUM:

RPMI 1640 with glutamine, without bicarbonate in MOPS (3N-Morpholino propane sulphonic acid), buffer sterilized by membrane filteration[96,88].

ANTI FUNGAL STOCK SOLUTION:

5ml stock solution prepared for each drug.

For water soluble drugs (eg Fluconazole)

Twofold dilutions of a water soluble antifungal agent is used, they may be prepared volumetrically in broth.

For water insoluble drugs-diluent DMSO

For example, to prepare for a broth microdilution test series containing a water-insoluble drug that can be dissolved in DMSO, for which the highest desired test concentration is 16µg/ml, first weigh 4.8 mg (assuming 100% potency) of antifungal powder and dissolve in 3.0 ml of DMSO. This will provide a stock solution at 1,600 µg/ml. Next prepare further dilutions of this stocks solution in DMSO. The solutions in DMSO will be further diluted 1:50 in the test medium and a further two fold when inoculated [96,99].

DRUG DILUTION:

To prepare 5 ml volumes of antifungal agent pipette 4.9 ml volumes of RPMI 1640 medium into each of 10 sterile test tubes. Now, using a single pipette add 0.1 ml of DMSO alone to one 4.9 ml lot of medium (control medium), then 0.1 ml of lowest (3.13 microgram /ml) drug concentration in DMSO, then 0.1 ml of the 6.25 μg/ml, concentration and continue in sequence up the concentration series, each time adding 0.1 ml volumes to 4.9 ml medium. These volumes were adjusted according to the total No. of test required. Because there will be 1:2 dilution of the drug when combined with the inoculums, the working antifungal solution are 2 fold more concentrated than the final concentration (NCCLS).

INOCULUM PREPARATION:

7-15 days old cultures grown on SDA at 25^{o}C was used. Mature colonies were covered with 10ml of sterile saline (0.85%). Growth scraped by sterile Pasteur pipette. Heavy particles allowed to settle for 15-20 minutes at room temperature. Supernatant was mixed with a vortex for 15 seconds. Turbidity of supernatant was adjusted spectrophotometrically to 530nm 65-70% absorbance. Each suspension was diluted 1:50 in RPMI 1640[98].

INOCULATING RPMI-1640 MEDIUM:

Each well will be inoculated on the day of test with 0.1 ml of 2x inoculums suspension. This step will dilute the drug concentration, inoculums densities, and solvent used to the final desired test concentration.

The growth control well contains 0.1ml of the corresponding diluted inoculums suspension and 0.1ml of the drug diluents without anti fungal agents.

TEST PROCEDURE:

Test was performed in sterile microtitre plates. Aliquots of 100µls of drug dilutions inoculated in 1-10 micotitre wells. Concentration of Fluconazole 0.01-64 µg/ml (Concentration of others 0.0039-16 µg/ml).Added 100 µl of inoculums into each well from 1 to 12.Growth control-tube 12 with inoculums and without antifungal drug [97,100,101].

INCUBATION:

All microdilution trays were incubated at 28^{o}C without agitation.

READING RESULTS:

The MIC was taken as the lowest concentration of antifungal agent that substantially inhibit growth of the organism as detected visually. For the conventional microdilution procedure, the growth in each MIC well is compared with that of the growth control with the aid of reading mirror.

Each micro titre well was then given a numerical score as follows.

No reduction in growth – 4

Slight reduction in growth or approximately 80% of growth control (drug free medium). – 3

Prominent reduction in growth or approximately 50% of growth control. 2

Slight growth or approximately 25% of growth control. - 1

Optically clear or absence of growth (NCCLS M -38A) - 0

STATISTICAL ANALYSIS:

Data thus collected on observing the specimen and patients details were entered in MS Excel spreadsheet. Data was analyzed using statistical package for social sciences (SPSS) software where descriptive tables were generated to demonstrate the findings. Chi square test was used to compare the proportions, Cohen's kappa coefficient was used to quantify the degree of agreement between the KOH positive and Culture positive cases. Validity of the KOH test was also performed to identify its sensitivity and specificity in accordance with the culture test. P value less than 0.05 was considered as statistical significance.

RESULTS

TABLE 1: AGE DISTRIBUTION (n=217).

S.NO	AGE GROUP	MALE	FEMALE	TOTAL(%)
1	1-10	7	8	15(6.96)
2	11-20	20	13	33(15.2)
3	21-30	34	20	54(24.88)
4	31-40	28	18	46(21.1)
5	41-50	27	9	36(16.5)
6	51-60	12	12	24(11.05)
7	>60	5	4	9(4.14)
	TOTAL	133(61.2%)	84(38.7%)	217
	(Chi Square) $X^2 = 5.692$ p=0.46			

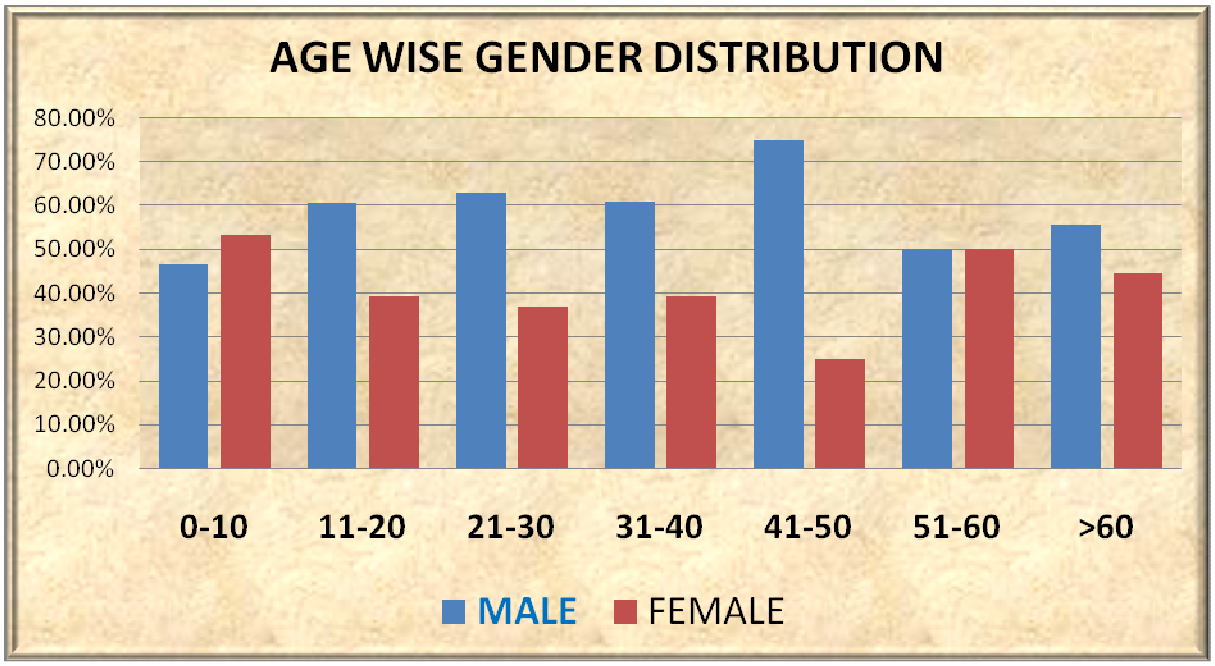
21 to 30 years (24.88%) age group was found to be more common, followed by 31 to 40 years (21.1%).The p value was 0.46 which was insignificant.

TABLE 2:GENDER DISTRIBUTION(n=217)

GENDER	**MALE**	**FEMALE**
FREQUENCY	133(61.2%)	84(38.7%)

There were 133 males(61.2%) and 84(38.7%) females. The male to female ratio is (1.58**:**1).

CHART I



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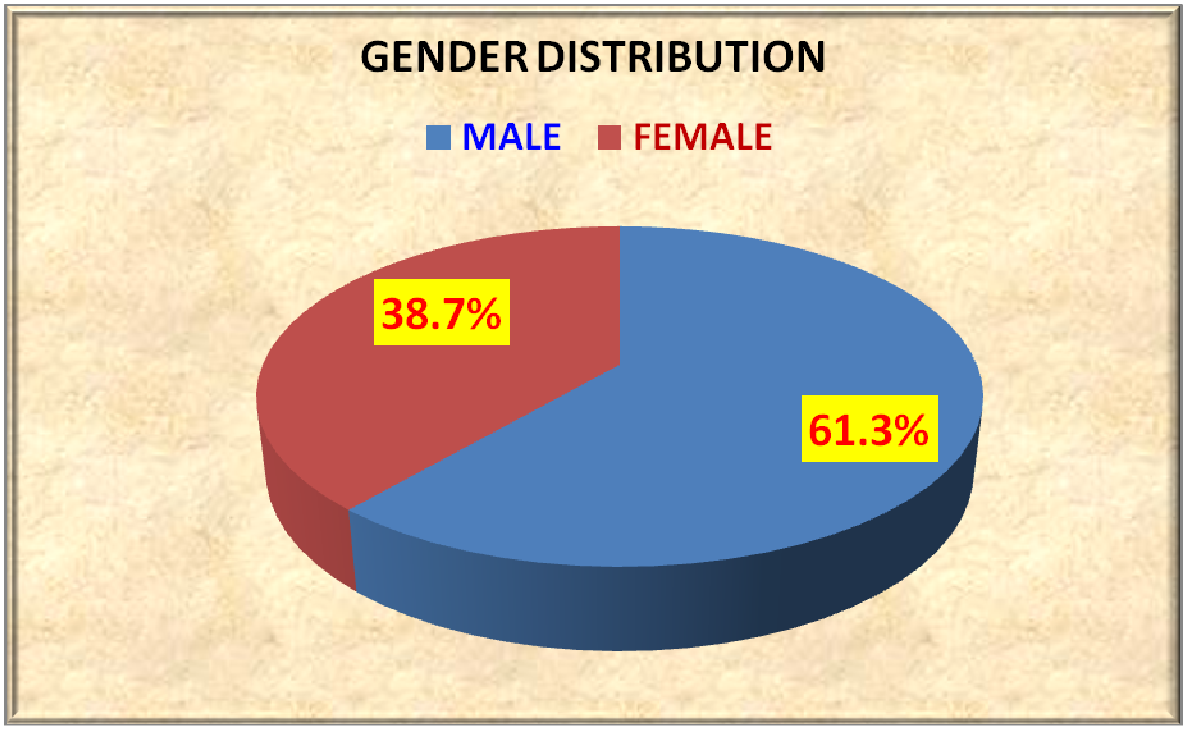


TABLE 3:RISK FACTORS(n=217)

S.NO	FACTORS	NO.OF CLASSES
1	CLOSE FAMILY CONTACTS	33(15.2%)
2	ANIMAL CONTACTS	41(18.89%)
3	DIABETICS MELLITUS	44(20.27%)
4	HYPERHIDROSIS	7(3.22%)
5	AGRICULTURER	35(16.12%)
6	HOSTLERS	25(11.5%)

Incidence of dermatophytes has been increasing on account of increasing in the incidence of diabetics. Most of the cases were engaged in occupation related to agriculture 35 (16.12%) followed by hostlers25 (11.5%)

TABLE 4:CLINICAL PRESENTATION OF DERMATOPHYTOSIS: (n=217)

SI NO	SPECIMEN	CLINICAL TYPE OF FUNGAL LESION	NO.OF CASES	PERCENTAGE
1	SKIN (n=139)	Tinea corporis	96	44.23%
		Tinea cruris	25	11.5%
		Tinea mannum	2	0.92%
		Tinea pedis	11	5.06%
		Tinea faciei	5	2.3%
2	HAIR(n=22)	Tinea capitis	21	9.67%
		Tinea barbae	1	0.46%
3	NAIL(n=56)	Tinea unguium	56	25.8%

Tinea corporis(44.23%) followed by Tinea unguium (25.8%) , Tinea cruris(11.5%),Tinea capitis (9.67%), Tinea pedis(5.06%) and Tinea faciei (2.3%)

TABLE 5:CORRELATION BETWEEN KOH EXAMINATION AND CULTURE OF THE ISOLATES:(n=217)

	10%KOH POSITIVE	**10%KOH NEGATIVE**	**TOTAL**
CULTURE POSITIVE	77(78.57%)	3(2.52%)	80(36.86%)
CULTURE NEGATIVE	21(21.42%)	116(97.45%)	137(63.13%)
TOTAL	98(45.16%)	119(54.83%)	217
	Coefficient correlation (r) =0.779, p=0.000*		

Out of 217 isolates 98(45.16%) was KOH positive and 119(54.83%) was KOH negative. 80(36.86%) isolates were culture positive and 137(63.13%) were culture negative for dermatophytes.

Sensitivity= 96.25%

Specificity=84.67%

The p value was 0.00 which was significant. So statistically 77.9% of the KOH positive samples were grown in culture and showed significant growth.

TABLE 6: PERCENTAGE OF DERMATOPHYTES ISOLATED IN SABOURAUDS DEXTROSE AGAR(n=217)

Culture positive	**80(36.8%)**
No growth	**137(63.1%)**

Out of 217 cases 80[36.8%] samples inoculated in Emmons modified sabourauds dextrose agar were shown significant growth with typical obverse and reverse pigmentation.

CHART III

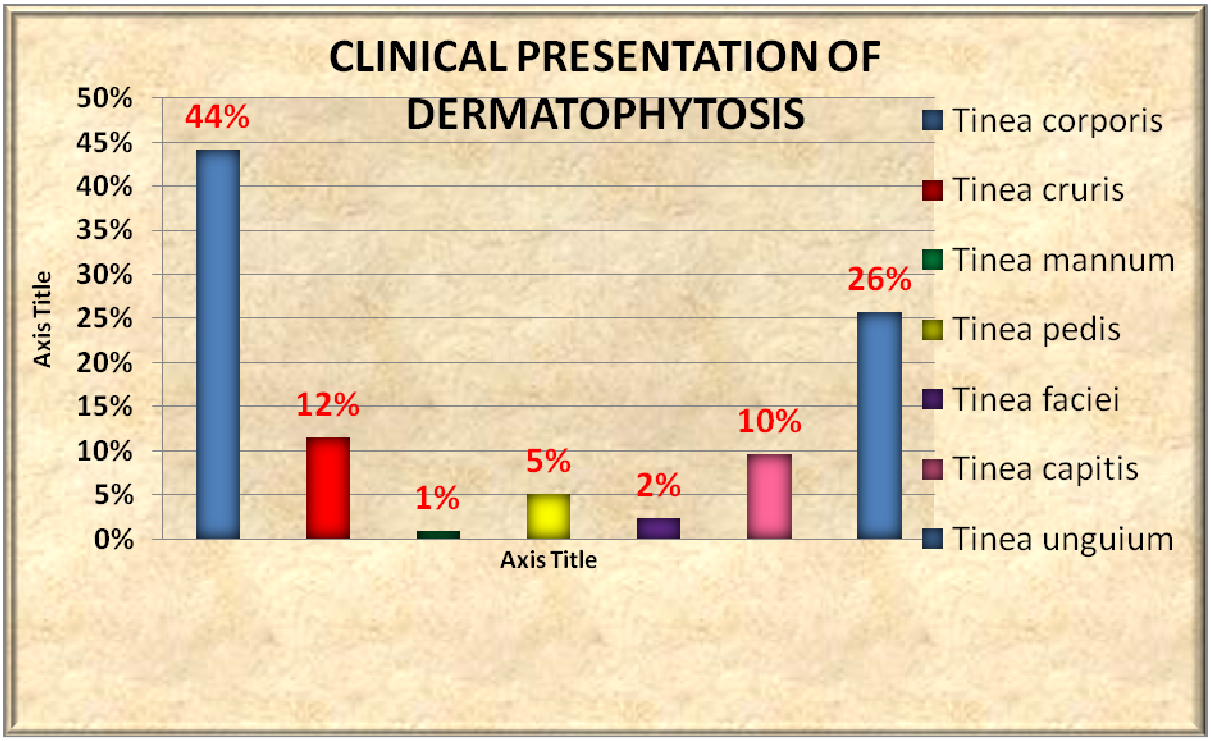


CHART IV

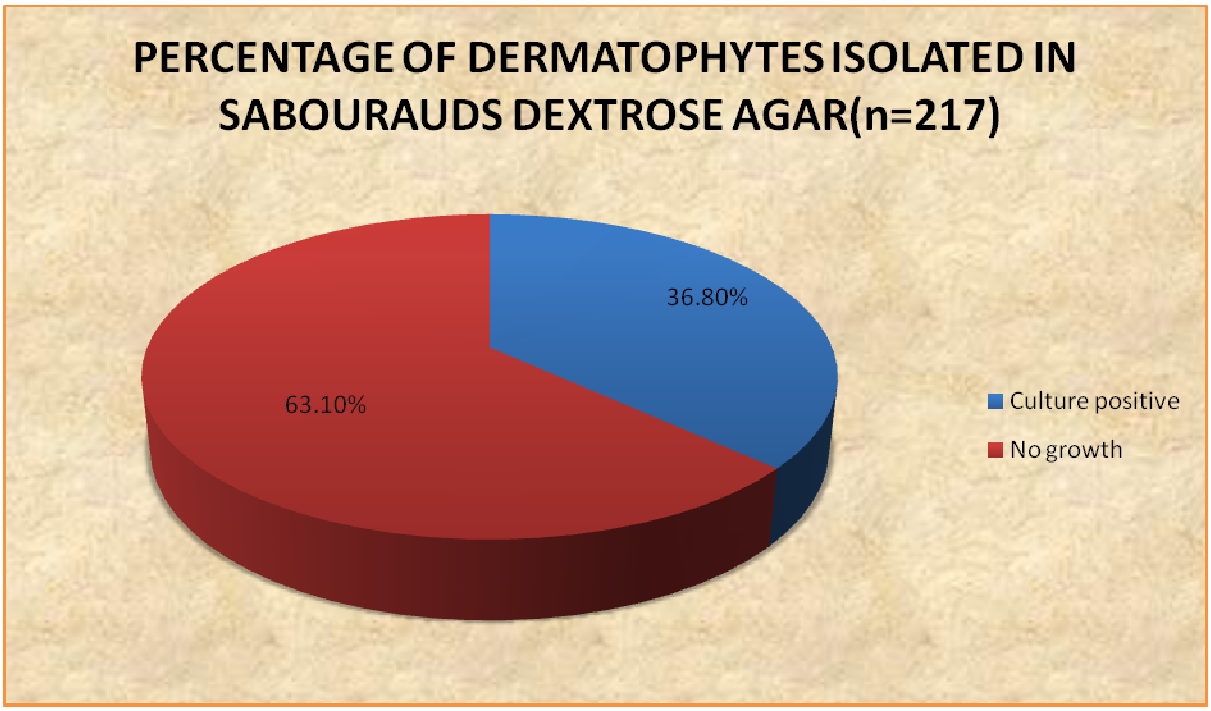


TABLE 7: FREQUENCY OF DERMATOPHYTIC SPECIES IN KMCH(n=80)

CLINICAL ISOLATES	NO.OF CASES
T.rubrum	63(78.75%)
T.mentagrophytes	7(8.75%)
T.verrucosum	6(7.5%)
E.floccosum	2(2.5%)
T.tonsurans	1(1.25%)
M.gypseum	1(1.25%)

Trichophyton was the most common genus in 77 out of 80 (96.25%) culture isolates. There was one isolate in microsporum and two isolates in epidermophyton. Total six species of dermatophytes were isolated on culture.

TABLE :8 DISTRIBUTION OF DERMATOPHYTIC ISOLATES IN CLINICAL SPECIMENS : (n=80)

SPECIMEN	ISOLATES	NO. OF CASE
SKIN	T.rubrum	57(71.25%)
	T.mentagrophytes	5(6.25%)
	T.verrucosum	2(2.5%)
	E.floccosum	1(1.25%)
HAIR	T.rubrum	1(1.25%)
	T.tonsurans	1(1.25%)
	M.gypseum	1(1.25%)
NAIL	T.rubrum	5(6.3%)
	T.verrucosum	4(5%)
	T.mentagrophytes	2(2.5%)
	E.floccosum	1(1.25%)

57 Trichophyton rubrum were isolated from skin samples and 1 from scalp hair and 5 from nail samples. Five Trichophyton mentagrophytes from skin and two from nail clippings were isolated.

CHART V

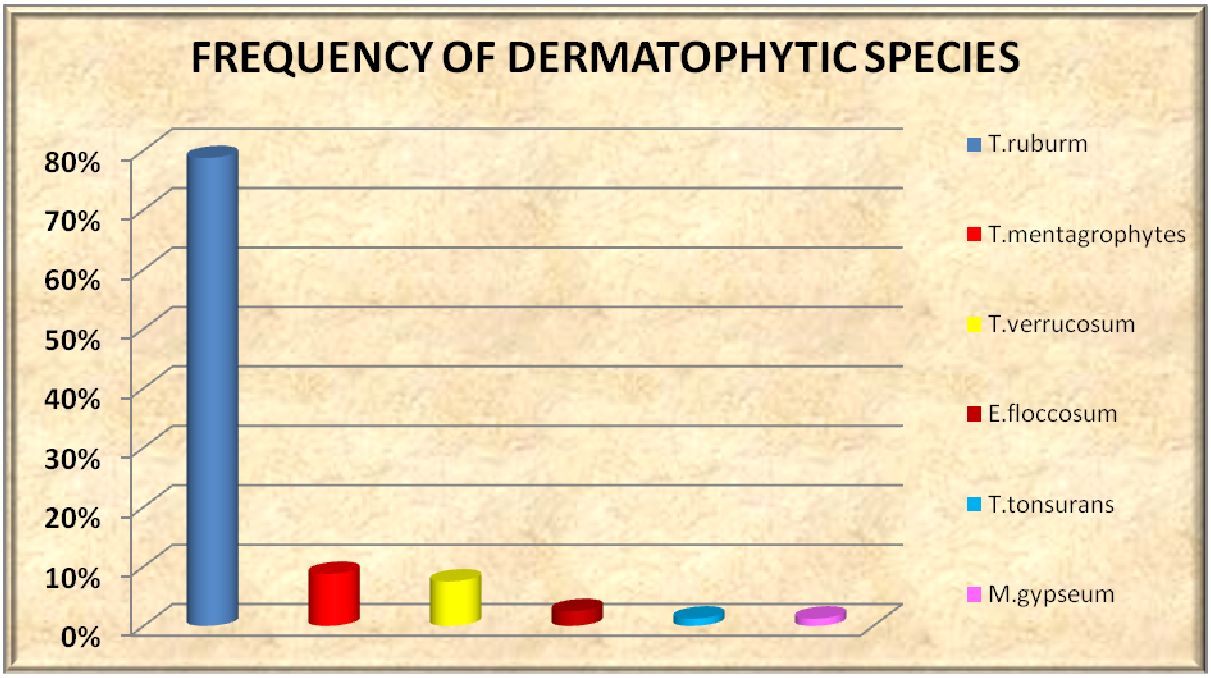


CHART VI

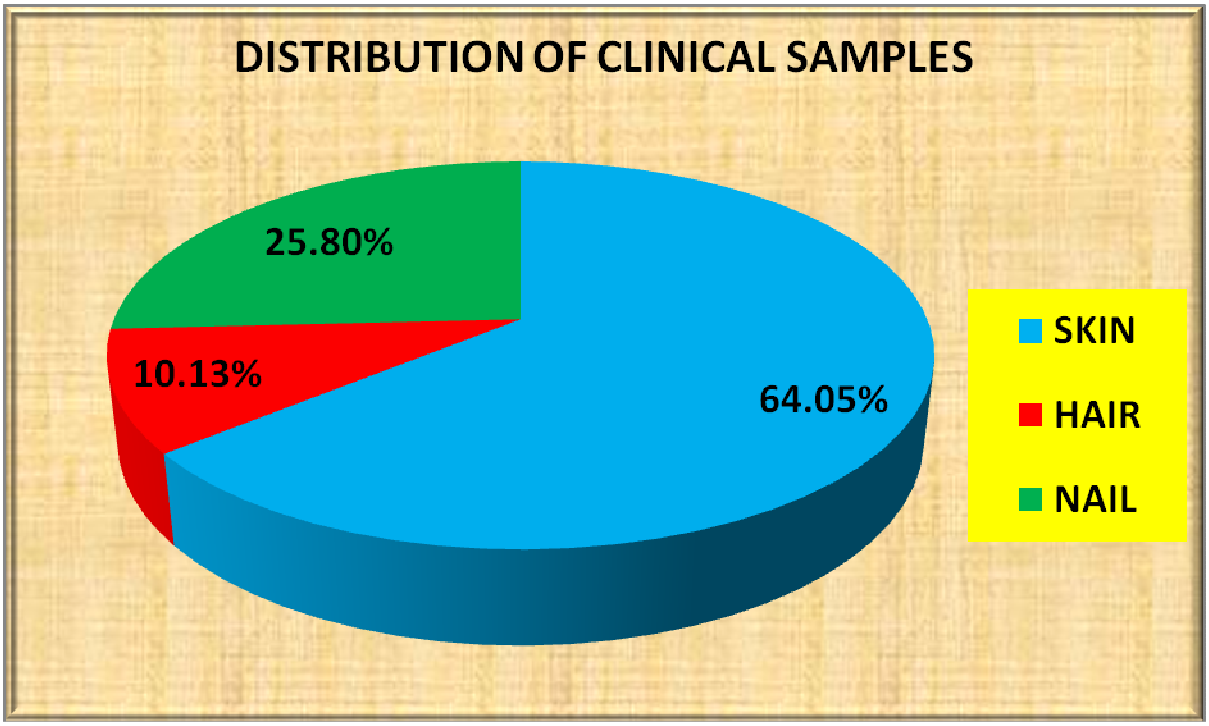


TABLE:9 DISTRIBUTION OF CLINICAL SAMPLES(n=217)

TOTAL NO. OF SPECIMEN	SKIN	HAIR	NAIL
217	**139(64.05%)**	**22(10.13%)**	**56(25.80%)**

Out of 217 clinical samples,139(64.05%) were skin scrapings, 22(10.13%) were hair samples and 56(25.80%) were nail samples.

TABLE: 10 DISTRIBUTION OF VARIOUS COMBINATIONS OF CLINICAL TYPES OF DERMATOPHYTES(n=217)

Clinical presentation	**Total no of cases**
Tinea corporis+ Tinea cruris	**9**
Tinea corporis + Tinea unguium	**3**
Tinea unguium+ Tinea pedis	**3**
Tinea corporis+ Tinea capitis	**2**
Tinea corporis+Tinea mannum	**1**
Tinea corporis+ Tinea faciei	**1**
Tinea corporis+ Tinea cruris+ Tinea mannum	**1**
Tinea corporis+ Tinea cruris+Tinea unguium	**1**
Tinea corporis+Tinea verscicolor	**2**

The most common clinical pattern observed was Tinea corporis with cruris followed byTinea corporis. Tinea capitis was the predominant dermatophyte infection in children.

TABLE:11 CLINICO – MYCOLOGICAL PROFILE OF DERMATOPHYTOSIS (N=80)

Clinical type	T.rubrum	T.mentagrophytes	T.verrucosum	T.tonsurans	E.floccosum	M.gypseum
Tinea corporis	32(40%)	4(5%)	1(1.25%)	-	-	-
Tinea cruris	12(15%)	1(1.25%)	-	-	1(1.25%)	-
Tinea pedis	7(8.75%)	-	-	-	-	-
Tinea barbae	1(1.25%)	-	-	-	-	-
Tinea faciei	4(5%)	-	-	-	-	-
Tinea manuum	1(1.25%)	-	1(1.25%)	-	-	-
Tinea unguium	5(6.25%)	2(2.5%)	4(5%)	_	1(1.25%)	_
Tinea capitis	1(1.25%)	-	-	1(1.25%)	-	1(1.25%)

Trichophyton rubrum (78.75%) followed by Trichophyton mentagrophytes (8.75%), Trichophyton verrucossum (7.5%), Epidermophyton floccosum (2.5%) , Trichophyton tonsurans (1.25%) and Microsporum gypseum (1.25%) were isolated.

.

TABLE12: HAIR PERFORATION TEST

DERMATOPHYTES	POSITIVE	NEGATIVE
Trichophyton rubrum(n=63)	0	63
Trichophyton mentagrophytes(n=7)	7	0

7 Trichophyton mentagrophytes shows positive hair perforation test and all the 63 Trichophyton rubrum isolated were negative for hair perforation test.

TABLE 13: UREASE TEST

DERMATOPHYTES	POSITIVE	NEGATIVE
Trichophyton mentagrophytes(n=7)	7	0
Trichophyton rubrum(n=63)	0	63

. Trichophyton mentagrophytes can hydrolyse urea thereby turned the medium to deep red while Trichophyton rubrum showed negative results. All the 7 Trichophyton mentagrophytes showed positive urease test and 63 Trichophyton rubrum were negative .

TABLE 14: DERMATOPHYTE TEST MEDIUM (n=217)

CLINICAL SAMPLES	DTM	SDA
SKIN	65	65
HAIR	3	3
NAIL	12	12

Out of the 217 samples 65 skin samples, 3 hair samples and 12 nail clippings were showed colour changes in dermatophyte test medium.

TABLE 15: MINIMAL INHIBITORY CONCENTRATION OF THE DRUG GRISOFULVIN

SPECIES	DRUG CONCENTRATIONS IN µg/ml											
	0.03	0.06	0.012	0.25	0.5	1	2	4	8	16	MIC 50	MIC 90
T.rubrum n=63	-	8	11	42	2	-	-	-	-	-	0.25	0.5
T.mentagrophytes n=7	1	2	1	3	-	-	-	-	-	-	0.12	0.25
T.verrucosum n=6	-	1	2	3	-	-	-	-	-	-	0.12	0.25
T.tonsurans n=1	-	-	-	1	-	-	-	-	-	-	0.25	0.25
E.floccosum n=2	-	1	-	1	-	-	-	-	-	-	0.06	0.25
M.gypseum n=1	-	1	-	-	-	-	-	-	-	-	0.06	0.06

MIC 50 and MIC 90 of Griseofulvin for the species isolated in this study are

Trichophyton rubrum was 0.25 and 0.5 µg/ml respectively

Trichophyton mentagrophytes was 0.12 and 0.25 µg/ml respectively

Trichophyton verrucosum was 0.12 and 0.25 µg/ml respectively

Trichophyton tonsurans was 0.25 and 0.25 µg/ml respectively

Epidermophyton floccosum was 0.06 and 0.25 µg/ml respectively

Microsporum gypseum was 0.06 and 0.06 µg/ml respectively

TABLE 16: MINIMAL INHIBITORY CONCENTRATION OF THE DRUG KETOCONAZOLE

SPECIES	DRUG CONCENTRATIONS IN µg/ml											
	0.03	0.06	0.012	0.25	0.5	1	2	4	8	16	MIC 50	MIC 90
T.rubrum n=63	-	4	39	14	6	-	-	-	-	-	0.12	0.5
T.mentagrophytes n=7	1	1	2	3	-	-	-	-	-	-	0.12	0.25
T.verrucosum n=6	-	2	1	-	3	-	-	-	-	-	0.06	0.5
T.tonsurans n=1	-	1	-	-	-	-	-	-	-	-	0.06	0.06
E.floccosum n=2	-	1	-	-	1	-	-	-	-	-	0.06	0.5
M.gypseum n=1	-	-	1	-	-	-	-	-	-	-	0.12	0.12

MIC 50 and MIC 90 of Ketoconazole for the species isolated in this study are

Trichophyton rubrum was 0.12 and 0.5 µg/ml respectively

Trichophyton mentagrophytes was 0.12 and 0.25 µg/ml respectively

Trichophyton verrucosum was 0.06 and 0.50 µg/ml respectively

Trichophyton tonsurans was 0.06 and 0.06 µg/ml respectively

Epidermophyton floccosum was 0.06 and 0.50 µg/ml respectively

Microsporum gypseum was 0.12 and 0.12 µg/ml respectively.

TABLE 17: MINIMAL INHIBITORY CONCENTRATION OF THE DRUG FLUCONAZOLE

SPECIES	DRUG CONCENTRATIONS IN µg/ml											
	0.03	0.06	0.012	0.25	0.5	1	2	4	8	16	MIC 50	MIC 90
T.rubrum n=63	-	-	-	-	-	42	18	3	-	-	1	4
T.mentagrophytes n=7	-	-	-	-	-	-	5	1	1	-	2	8
T.verrucosum n=6	-	-	-	-	-	-	4	2	-	-	2	4
T.tonsurans n=1	-	-	-	-	-	-	1	-	-	-	2	2
E.floccosum n=2	-	-	-	-	-	-	-	2	-	-	4	4
M.gypseum n=1	-	-	-	-	-	-	-	1	-	-	1	1

MIC 50 and MIC 90 of Fluconazole for the species isolated in this study are

Trichophyton rubrum was 1 and 4 μg/ml respectively

Trichophyton mentagrophytes was 2 and 8 μg/ml respectively

Trichophyton verrucosum was 2 and 4 μg/ml respectively

Trichophyton tonsurans was 2 and 2 μg/ml respectively

Epidermophyton floccosum was 4 and 4 μg/ml respectively

Microsporum gypseum was 1 and 1 μg/ml respectively

TABLE 18: MINIMAL INHIBITORY CONCENTRATION OF THE DRUG ITRACONAZOLE

SPECIES	DRUG CONCENTRATION IN (µg/ml)										
	0.0075	0.015	0.03	0.06	0.12	0.25	0.5	1	2	MIC 50	MIC 90
T.rubrum n=63	-	-	5	28	23	7	-	-	-	0.12	0.25
T.mentagrophytes n=7	-	3	2	-	2	-	-	-	-	0.03	0.12
T.verrucosum n=6	-	-	1	3	-	2	-	-	-	0.06	0.25
T.tonsurans n=1	-	-	-	1	-	-	-	-	-	0.06	0.06
E.floccosum n=2	-	-	1	1	-	-	-	-	-	0.03	0.06
M.gypseum n=1	-	-	-	1	-	-	-	-	-	0.06	0.06

MIC 50 and MIC 90 of Intraconazole for the species isolated in this study are

Trichophyton rubrum was 0.12 and 0.25 µg/ml respectively

Trichophyton mentagrophytes was 0.03 and 0.12 µg/ml respectively

Trichophyton verrucosum was 0.06 and 0.25 µg/ml respectively

Trichophyton tonsurans was 0.06 and 0.06 µg/ml respectively

Epidermophyton floccosum was 0.03 and 0.06 µg/ml respectively

Microsporum gypseum was 0.06 and 0.06 µg/ml respectively

CLINICAL PRESENTATION

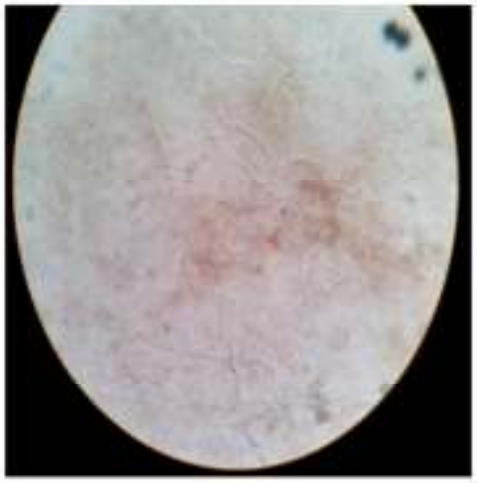
TINEA CORPORIS

TINEA CRURIS

TINEA CAPITIS

TINEA UNGUIUM

KOH MOUNT

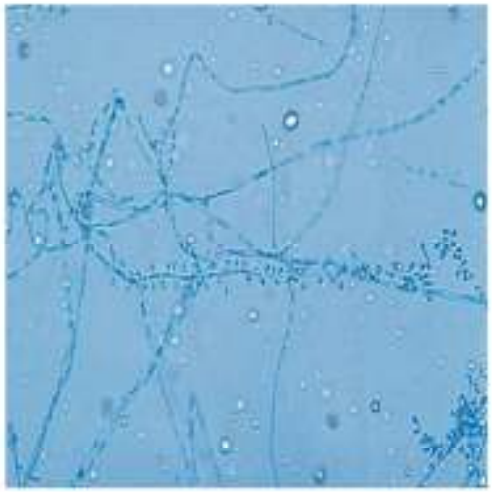


DERMATOPHYTE TEST MEDIUM

TRICHOPHYTON RUBRUM

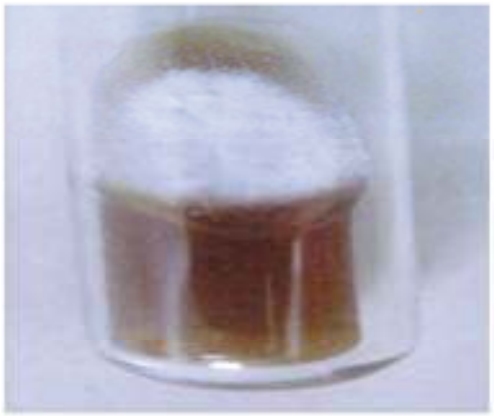
GROWTH ON SDA

LPCB MOUNT

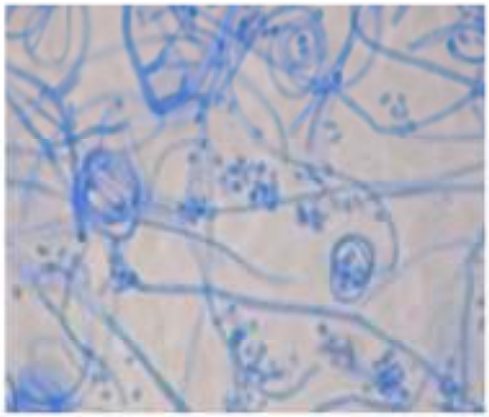


TRICHOPHYTON MENTAGROPHYTES

GROWTH ON SDA



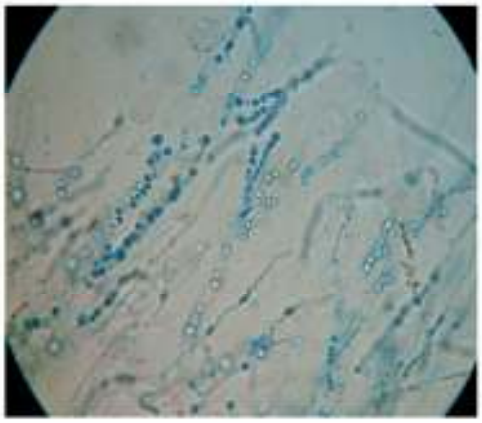
LPCB MOUNT



TRCHOPHYTON VERRUCOSUM

GROWTH ON SDA

LPCB MOUNT



TRICHOPHYTON TONSURANS

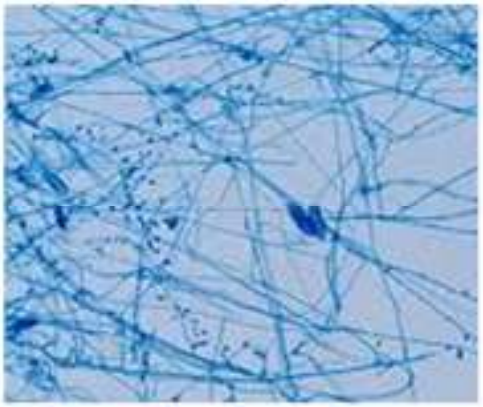
GROWTH ON SDA

LPCB MOUNT

MICROSPORUM GYPSEUM

GROWTH ON SDA

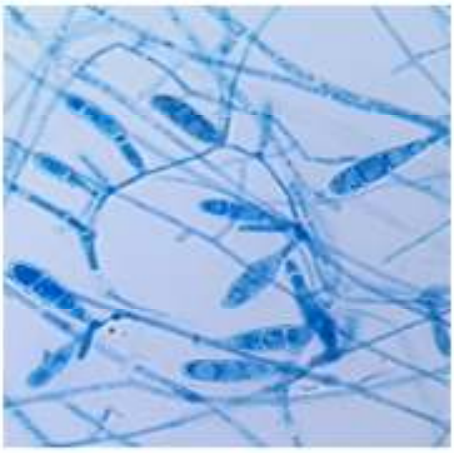
LPCB MOUNT



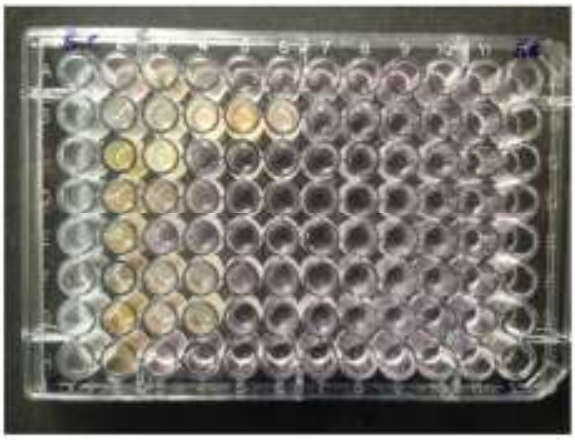
EPIDERMOPHYTON FLOCCOSUM

GROWTH ON SDA

LPCB MOUNT



MICROBROTH DILUTION METHOD



DISCUSSION:

This study aims to isolate the dermatophytes causing infection of skin, hair, nail and to speciate the isolates of dermatophytes and to determine the antifungal susceptibility of the isolates obtained from dermatology Out patient department in Kilpauk Medical college.

Out of 217 patients, the maximum number of patients found in the age group of 21 to 30 years were 24.88%, followed by 31 to 40 years(21.1%). The higher incidence in young males could be due to greater physical activity and increased sweating. Similar results were shown in the studies of Peerapur et al[1], Sumam singh et al[3] 45.38%, clarissa J.Lyngdoh cet al[4] showed 34.4%, Agarwal et al[5] showed 30.3% ,Smita Sarma et al[6] showed 39% and Surendran KAK et al[7] showed 44% .In this study the youngest patient was two years male child and the eldest was 77 years old male.

There were 133 males (61.2%) and 84(38.7%) females. The male to female ratio is 1.58**:**1. Males outnumbered females due to the prevailing social stigma, illiteracy, poor personal hygiene and environmental conditions. Veer et al[9] showed the males to females ratio were 1.8:1, similar results were obsevered in Arun Vyas et al, Agarwal US et al[8] and peerapur et al[1]

In the present study, 44 patients were presented with Diabetes Mellitus (20.27%), 41 had a history of animal contacts (18.89%), 33 patients had a family history of dermatophytic infections (15.2%). Most of the cases were engaged in occupation related to agriculture (16.5%) followed by Hostlers (11.5%). Reduced peripheral circulation, diabetes and poor personal hygiene were responsible for the increase in dermatophytic infections as per the study of P.Veer et al[9]

The common clinical types of dermatophytoses that present to us were Tinea corporis(44.23%) followed by Tinea cruris(11.5%) which concurs with the studies of Suman singh et al[3] and Agarwal U.S et al[5]

The incidence of Tinea capitis (9.67%) in this study was comparable with Suman singh et al[3] study. Tinea capitis is less common in India than in other countries, this may be attributable to the use of hair oils used by Indians and have been shown to have an inhibitory effect on dermatophytosis [81].

The incidence of Tinea pedis (5.06%) in this study was comparable with Venkatesan et al[11] (5.6%). The predominance of Tinea pedis in western countries could be because of the regular use of shoes and shocks, resulting in

conditions like dampness and warmth of the body thereby facilitating the skin surface for the growth of dermatophytes.

Out of 217 isolates 98(45.16%) was KOH positive and 119(54.83%) was KOH negative. 80(36.86%) isolates were culture positive and 137(63.13%) were culture negative for dermatophytes .In this study three of the culture positive samples showed no fungal filaments on direct KOH mount because of the fungus in an inactive sporulating phase difficult to be seen by microscopy but able to grow in appropriate medium. Out of the culture negative cases 21 showed fungal elements on KOH mount but failed to grow in culture. Surenderan et al[77] study showed similar results.This could be due to non viability of the fungi prior to inoculation and inappropriate use of antimycotic treatment and self medication before sampling.

Out of 217 cases 80 [36.8%] cases were culture positive. The isolation rate varying from 7% to 49% in other studies. Suman singh et al [3] showed 44.62% culture positivity, Clarissa et al[4] showed 29.3%, Surenderan et al[7] showed 39%. The difference in these rates among different studies may be due to factors involved in the collection, transport, inoculation of specimen, culture condition severity, type and stage of the disease and the effect of anti fungal agents.

Among the 80 culture positive isolates, 65 where obtained from skin scrapping, 3 from scalp hair and 12 from nail clippings.57 Trichophyton rubrum were isolated from skin samples and 1 from scalp hair and 5 from nail. Five Trichophyton mentagrophytes from skin and two from nail clippings were isolated. Trichophyton rubrum was the chief isolate from skin samples similar to Suman Singh et al[3] and Clarissa et al[4] study. Tinea mentagrophytes were the second common isolate from glabarous skin from the body.

Out of 217 clinical samples,139(64.05%) were skin scrapings, 22(10.13%) were from hair specimens and 56 (25.8%) were from nail clippings ansd subungal debris.More isolates were obtained from skin scrapings and least from nail and hair samples . P Kannan et al[12] studies were showed similar results.

The most common clinical pattern observed was Tinea corporis with cruris followed by Tinea corporis, which was in accordance with Bindu et al[13] study. But in other study in northeast India Grover et al[30] showed Tinea pedis(29.2%) as the common pattern followed by Tinea cruris(26.2). Clarissa et al[4] showed Tinea capitis as the predominant dermatophyte infection in children below 15 years[103] .Tinea corporis with Tinea unguium

and Tinea unguium with mannum clinical pattern were also recorded in this present study.

All three genera of dermatophytes such as Trichophyton, Epidermophyton and Microsporum have been isolated as the causative agent in this study. Totally six species of dermatophytes were isolated. Clarissa et al [4] and Surenderan et al[7] showed the similar results.

Trichophyton rubrum was found to be the commonest etiological agent (78.75%) isolated from tinea corporis , tinea pedis, tinea mannum, tinea faciei, tinea barbae, tinea capitis, tinea cruris and tinea unguium , followed by Trichophyton mentagrophytes (8.75%), Trichophyton verrucossum (7.5%), Epidermophyton floccosum (2.5%) , Trichophyton tonsurans (1.25%) and Microsporum gypseum (1.25%).

As accordance with Suman singh et al[3] Trichophyton rubrum was the common isolate from tinea corporis.

Trichophyton rubrum was the predominant dermatophyte isolated from Tinea cruris but Suman singh et al[3] showed Epidermophyton floccosum as the chief isolate[30].

In accordance with P Veer et al[9], Trichophyton rubrum was the common isolate from tinea unguium.

The invitro hair perforation test is used to differentiate between Trichophyton rubrum and Trichophyton mentagrophytes by the ability of the dermatophyte species to produce wedge shaped perforations in the blonde hair[19]. All the 7 Trichophyton mentagrophytes shows positive hair perforation test.

The urease test was done on Christensen's urease medium and was also in urease broth to differentiate Trichophyton mentagrophytes from Trichophyton rubrum[8,25,31,32]. Trichophyton mentagrophytes can hydrolyse urea thereby turned the medium to deep red while Trichophyton rubrum showed negative results. All the 7 Trichophyton mentagrophytes shows positive urease test.

Dermatophyte test medium was used for presumptive identification of dermatophytes[28].Most of the dermatophytes species produce red colour due

to liberation of alkaline metabolites.The medium was incubated at 25°c and the change in colour occurs within 3 to 6 days.

ANTI FUNGAL SUSCEPTIBILITY BY MICRO BROTH DILUTION METHOD

In the present study the MIC range, MIC 50 and MIC 90 for the drug Griseofulvin was found to be 0.03-0.5, 0.25 and 0.5 respectively. The MIC range MIC 50 and MIC 90 for the drug Ketoconazole was found to be 0.03-0.5, 0.06 and 0.5 repectively. The MIC range, MIC 50 and MIC 90 for the drug

Fluconazole was found to be 1-8, 2 and 4 respectively. The MIC range , MIC 50 and MIC 90 for the drug Itraconazole was found to be 0.015-0.25, 0.06 and 0.12 respectively.

The multicenter study by M.A. Ghannoum et al[100] determine the inter and intra laboratory reproducibility of MIC testing by micro broth dilution method of the common dermatophytic infection,shows an increase in the MIC

values of Griseofulvin and Fluconazole . The present study correlates with the studies conducted by Fernandez Torres et al[14], C.J.Jessup et al[15].

Even though Norries et al[100] and C.J.Jessup et al[50] studies established the inoculums size, optimum condition, optimum medium for conidial formation , incubation time duration and end point determination but standard reference method for antifungal susceptibility testing of dermatophytic infection is lacking. For this study the microdilution method was chosen because of its conveniency, reproducibility and greater ease of performance. Fluconazole showed a higher MIC values in the range of 1-8µg/ml. Itraconazole showed the lowest MIC values by micro broth dilution method and found to be the most potent drug.

The increased incidence and availability of various new drugs for dermatophytic infection in the last two decades emphasis a reference susceptibility testing method ,which aids the clinician to select the appropriate drugs for the management of dermatophytic infection

SUMMARY

- 217 Samples from suspected Dermatophytic patients were collected and processed. The Male/Female ratio was 1.58:1. Most of the cases were seen between 21 to 30 years.

- Diabetes Mellitus (20.27%), contacts with animals (18.89%), and close family contacts with dermatophytic infections (15.2%) were the common identified risk factors. Most of the cases were engaged in occupation related to agriculture (16.5%) followed by Hostlers (11.5%).

- Out of 217 samples, 45.16% were KOH positive and 36.86% were Culture positive. 2.52% were KOH negative but culture positive. 21.42% were KOH positive but culture negative. 78.54% were both KOH and culture positive.

- Samples were skin scrapings, Nail clipping and hairs. 80 (36.86%) cases were culture positive. In which 96.25% Trichophyton species, 2.5% Epidermophyton species and 1.25% Microsporum species were isolated.

- In Tinea corporis predominant isolates was Trichophyton rubrum (71.25%) followed by Trichophyton mentagrophytes (6.25%), Trichophyton verrucosum (2.5%) and Epidermophyton floccosum (1.25%). Trichophyton rubrum was the predominant isolate in Tinea pedis, Tinea barbae, Tinea faciei, Tinea cruris, Tinea capitis, Tinea mannum and Tinea unguium.

- Trichophyton rubrum, Trichophyton tonsurans and Microsporum gypseum were isolated in equal proportion in Tinea capitis. In Tinea unguium the predominant isolates was Trichophyton rubrum (6.3%) followed by Trichophyton verrucosum (5%), Trichophyton mentagrophytes (2.5%), and Epidermophyton floccosum (1.25%).

- Anti fungal susceptibility testing was performed by micro broth dilution method for Griseofulvin, Ketoconazole, Fluconazole and Itraconazole.

- The MIC range for Griseofulvin by microbroth dilution method was 0.03µg/ml- 0.50 µg/ml. The MIC range for Ketoconazole by microbroth dilution method was 0.03µg/ml- 0.50 µg/ml. The MIC

range for Fluconazole by microbroth dilution method was 1µg/ml- 8 µg/ml. The MIC range for Itraconazole by microbroth dilution method was 0.015µg/ml- 0.25 µg/ml.

- Fluconazole showed a higher MIC values when compared to other anti fungal drugs. Itraconazole showed the lowest MIC values by micro broth dilution method and found to be the most potent drug.

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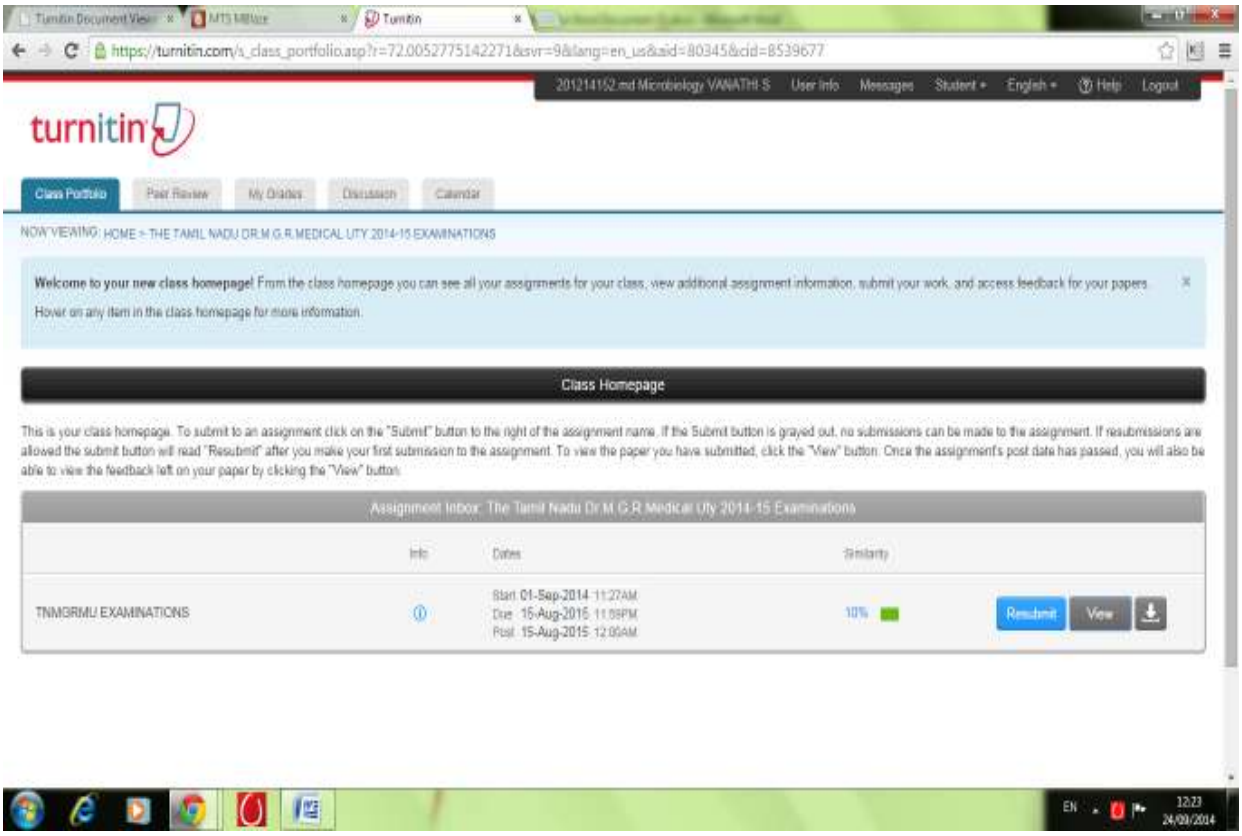
CONCLUSION

- 217 clinically suspected cases of dermatophytes were subjected to mycological study.
- Male: Female ratio was 1.58 : 1
- Most of the cases were seen between 21 – 30 years of age group. This is due to increased activity and post pubertal changes.
- 80 (36.86%) isolates were culture positive.
- All 3 genera of dermatophytes were isolated. Trichophyton (96.2%) species were the predominant isolates followed by Epidermophyton (2.25%) and Microsporum (1.25%)
- Trichophyton rubrum was the predominant species isolated from Tinea corporis, Tinea cruris, Tinea unguium and Tinea capitis.
- The other dermatophytes isolated were Trichophyton mentagrophytes, Trichophyton verrucosum, Trichophyton tonsurans Epidermophyton floccosum, and Microsporum gypseum.
- In vitro hair perforation test and Urease test helps to differentiate Trichophyton mentagrophytes from Trichophyton rubrum.

- The minimal inhibitory concentration of Griseofulvin was 0.03-0.5µg/ml. The minimal inhibitory concentration of Itraconazole was 0.015-0.25µg/ml.

- Fluconazole showed a higher MIC value when compared to other drugs by micro broth dilution method. Itraconazole was found to be the most effective of all the drugs tested.

- Specific identification of the dermatophytic species and timely institution of appropriate antifungal therapy based on the prevailing sensitivity pattern could be of immense value.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ISOLATION, SPECIATION AND ANTI FUNGAL SUSCEPTIBILITY OF
DERMATOPHYTIC INFECTION IN A PATIENT ATTENDING A
TERTIARY CARE HOSPITAL
INTRODUCTION:

INSTITUTIONAL ETHICAL COMMITTEE
GOVT.KILPAUK MEDICAL COLLEGE,
CHENNAI-10
Ref.No.10499/ME-I/Ethics/2012 Dt:06.12.2012.
CERTIFICATE OF APPROVAL

The Institutional Ethical Committee of Govt. Kilpauk Medical College, Chennai reviewed and discussed the application for approval "A Study on Isolation, Speciation and Antifungal Susceptibility of Dermatophytic infection in patients attending a Tertiary care Hospital" for dissertation purpose submitted by Dr.S.Vanathi, I'sy year MD, Microbiology PG student, Kilpauk Medical College, Chennai.

The Proposal is APPROVED.

The Institutional Ethical Committee expects to be informed about the progress of the study any Adverse Drug Reaction Occurring in the Course of the study any change in the protocol and patient information /informed consent and asks to be provided a copy of the final report.

13/12/12
CHAIRMAN,
Ethical Committee
Govt.Kilpauk Medical College,Chennai

PROFORMA

NAME:

AGE /SEX:

OP NUMBER:

ADDRESS:

OCCUPATION:

H/O PRESENT ILLNESS:

DURATION

SYMPTOMS

SIGNS

RISK FACTORS:

FAMILY CONTACTS: **PET ANIMALS:**

PAST HISTORY:

TREATMENT HISTORY:

COMORBID CONDITIONS:

SPECIMEN:

SKIN

HAIR

NAIL

CLINICAL DIAGNOSIS:

ஒப்புதல் படிவம்

ஆய்வு செய்யப்படும் தலைப்பு

நிறுவனம்/மருத்துவக் கல்லூரி

பங்கு பெறுபவரின் பெயர் :

பங்கு பெறுபவரின் வயது :

பங்கு பெறுபவரின் எண் :

பங்கு பெறுபவர் இதனை (✓) குறிக்கவும்.

மேலே குறிப்பிட்டுள்ள மருத்துவ ஆய்வின் விவரங்கள் எனக்கு விளக்கப்பட்டது. என்னுடைய சந்தேகங்களை கேட்கவும், அதற்கான நகுந்த விளக்கங்களை பெறவும் வாய்ப்பளிக்கப்பட்டுள்ளது என அறிந்து கொண்டேன். ☐

நான் இவ்வாய்வில் தன்னிச்சையாக தான் பங்கேற்கிறேன், எந்த காரணத்தினாலோ எந்த சட்டசிக்கலுக்கும் உட்படாமல் நான் இவ்வாய்வில் இருந்து விலகி கொள்ளலாம் என்றும் அறிந்தும் கொண்டேன். ☐

இந்த ஆய்வு சம்பந்தமாகவோ, இதை சார்ந்து மேலும் ஆய்வு மேற்கொள்ளும் பொருட்டோ இந்த ஆய்வில் பங்கு பெறும் மருத்துவர் என்னுடைய மருத்துவ குறிப்புகளை பார்ப்பதற்கு என் அனுமதி தேவையில்லை என அறிந்து கொள்கிறேன். ☐

இந்த ஆய்வில் மூலம் கிடைக்கும் தகவலையோ, முடிவையோ பயன்படுத்திக் கொள்ள மறுக்கமாட்டேன். ☐

இந்த ஆய்வில் பங்கு கொள்ள ஒப்புக் கொள்கிறேன். இந்த ஆய்வை மேற்கொள்ளும் மருத்துவ அணிக்கு உண்மையுடன் இருப்பேன் என்றும் உறுதியளிக்கிறேன். ☐

பங்கேற்பவரின் கையொப்பம் _____ இடம் _____ தேதி _____

இடம் _____ தேதி _____

பங்கேற்பவரின் பெயர் மற்றும் விலாசம்
சாட்சியாளரின் கையொப்பம்

இடம் _____ தேதி _____
சாட்சியாளரின் பெயர் மற்றும் விலாசம்

ஆய்வாளரின் கையொப்பம்
இடம் _____ தேதி _____

ஆய்வாளரின் பெயர் _____

APPENDIX

POTASSIUM HYDROXIDE MOUNTS

The aqueous Potassium hydroxide (KOH) digests proteins debris and dissolves cement substance, which holds keratinized cells together. It is prepared from the following ingredients:-

Potassium hydroxide	10gm
Glycerol	10ml
Distilled Water	80ml

To solution of 10% KOH, 10% glycerol is added to prevent drying. Mix these ingredients properly and store solution at room temperature. The concentration of KOH can be increased depending upon nature of clinical material, as solid specimens require 10-40% KOH solution, which helps as best cleansing agent of fungi (KOH-20gm, DMSO-40ml).

LACTOPHENOL COTTON BLUE STAIN

The Lactophenol Cotton Blue (LPCB) mount is used to study morphological features of fungal isolates. It is of two types:-

PLAIN LPCB

It contains following ingredients:-

Melted Phenol : 20ml

Lactic Acid : 20ml

Glycerol : 40ml

Cotton Blue : 0.05gm

Distilled Water : 20ml

Mix all regents properly and dissolve 0.05gm of Cotton Blue stain in distilled water before mixing with remaining reagents. The phenol acts as disinfectant, lactic acid preserves morphology of fungi and glycerol is hygroscopic agent which prevents drying. The Cotton Blue stains outer wall of fungus.

SUBOURAUD DEXTROSE AGAR WITH ANTIBIOTICS

Composition of Sabouraud's Dextrose Agar (Emmon's Modification)

Dextrose : 20gm

Peptone : 10g

Agar : 20g

Distilled Water : 1000ml

Final pH : 6.9

The ingredients by dissolved by boiling 50 miligram per Ltr of chloramphenicol was added and 500 milligrams per ltr of Cycloheximide. Chloramphenicol was dissolved in 10ml of 95% Ethanol and added to boiling medium. Chloramphenicol was dissolved in 10ml of acetone and added to the boiling medium. Autoclave 121^0 for 15 minutes, Dispense in Tubes and allow Cooling in slanted position.

Brain-Heart Infusion Agar

Composition

Brain Heart Infusion: 37gms

Glucose : 20gms

L-Cysteine Hydrochloride : 1gm

Agar : 20gms

Distilled Water: 900 ml

Dissolve the ingredients by boiling. Dispense into Scru-captubes and auto clave 121^0 C for 15 minutes. The antibiotics cycloheximide, chloramphicol and gentamicin are added to this medium as for SDA. Cool in slanted position stored in refrigerator pH is adjusted to 6.7.

RPMI 1640 medium:

Commercially purchased RPMI 1640 media supplement with 0.3g of L-glutamate per litre without sodium bicarbonate.Dissolve the powder in

nuclease free water.The medium was sterilized by filtering through a sterile membrane filter with a porosity of 0.22 microns. The pH was adjusted to 7.0. MOPS buffer was used.

POTATO DEXTROSE AGAR

For convenience this medium is generally prepared from dehydrated commercial preparation according to the instruction of the manufacturer. Alternatively this medium may be prepared.

From raw materials as follows:

Potato : 200g

Dextrose : 20g

Agar : 20g

Water: 1 lit

Scrub but do not peel the potato & cut into 12ml cubes, Boil 200g in 1 lit of water for 60 minutes.

Squeeze as much of the pulp as possible through a fine sieve. Add agar and boil till dissolved. Add dextrose and make up to 1 liter. Dispense in required amounts taking care to keep solids in suspension. Autoclave at 121^0C

for15 minutes. Cool to 50^0 C & pour into tubes and allowed to cool in slanted position.

BLOOD AGAR

It contains the following ingredients:

Agar base : 40gm

Sheep blood : 50ml

Distilled water : 1000ml

MODIFIED CHRISTENSEN'S MEDIUM FOR UREASE

Hydrolysis:

Peptone : 1.0g

NaCl :5.0g

KH2PO4 :2.0g

Glucose: 5.0g

Agar:20.0g

Distilled Water: 1000 ml

After dissolving the above ingredients by heating 5 ml of phenol red solution (0.2% in 50% alcohol) was added after which autoclaved at 121°C for 15 minutes. On cooling 100 ml of urea (20% aqueous solution sterilized by solution) was added and medium was poured into slopes.

TABLE 1: SCHEME FOR PREPARING DILUTION SERIES OF WATER-INSOLUBLE ANTIFUNGAL AGENTS TO BE USED IN BROTH DILUTION SUSCEPTIBILITY TESTS

Antimicrobial Solution						
Step	Concentration (µg/ml)	Source	Volume + Solvent (ml) e.g. DMSO	= Intermediate concentration (µg/ml)	= Final Concentration at 1:50 (µg/ml)	Log
1	1600	Stock		1600	32	4
2	1600	Stock	0.5 0.5	800	16	3
3	1600	Stock	0.5 1.5	400	8	2
4	1600	Stock	0.5 3.5	200	4	1
5	200	Step 4	0.5 0.51	100	2	0
6	200	Step 4	0.5 1.5	50	1	-1
7	200	Step 4	0.5 3.5	25	0.5	-2
8	25	Step 7	0.5 0.5	12.50	0.25	-3
9	25	Step 7	0.5 1.5	6.25	0.125	-4
10	25	Step 7	0.5 3.5	3.13	0.0625	-5

Table 2: SCHEME FOR PREPARING DILUTION OF WATER-SOLUBLE ANTIFUNGAL AGENTS TO BE USED IN BROTH SUSCEPTIBILITY TESTS

Antimicrobial Solution						
Step	Concentration (µg/ml)	Source	Volume + Medium (ml) + (ml)	= Intermediate concentration (µg/ml)	= Final Concentration at 1:50 (µg/ml)	Log
1	5120	Stock	1 7	640	128	6
2	640	Step 1	1 1.0	320	64	5
3	640	Step 1	1 3.0	160	32	4
4	160	Step 3	1 1.0	80	16	3
5	160	Step3	0.5 1.5	40	8	2
6	160	Step 3	0.5 3.5	20	4	1
7	20	Step 6	1 1.0	10	2	0
8	20	Step 6	0.5 1.5	5	1	-1
9	20	Step 6	0.5 3.5	2.5	0.5	-2
10	2.5	Step 9	1 1.0	1.25	0.25	-3
11	2.5	Step 9	0.5 1.5	0.625	0.125	-4
12	2.5	Step 9	0.5 3.5	0.0625	0.0625	-5

ABBREVIATION

1.	KOH	-	Potassium Hydroxide
2.	LPCB	-	Lacto Phenol Cotton Blue
3.	DTM	-	Dermatophyte test medium
4.	SDA	-	Sabouraud's Dextrose Agar
5.	MIC	-	Minimum Inhibitory Concentration
6.	CLSI	-	Clinical and Laboratory Standards Institute
7.	RPMI	-	Roswell Park Memorial Institute
8.	NCCLS	-	National Committee for Clinical Laboratory Standard
9.	SPSS	-	Statistical Package for Social Sciences
10.	MOPS	-	3N- Morpholino propane sulphonic acid
11.	DMSO	-	Dimethyl Sulphoxide
12.	NA	-	Nutrient Agar
13.	PCR	-	Polymerase Chain Reaction
14.	KMCH	-	Kilpauk Medical College & Hospital

KEY TO MASTER CHART

M	-	Male
F	-	Female
KOH	-	Potassium Hydroxide
DTM	-	Dermatophyte Test Medium
MIC	-	Minimum Inhibitory Concentration
MIC – Keto	-	MIC – Ketoconazole
MIC – Itra	-	MIC – Itraconazole
MIC – Griseo	-	MIC - Griseofulvin
MIC- Fluco	-	MIC - Fluconazole
T.corporis	-	Tinea corporis
T.pedis	-	Tinea pedis
T.unguim	-	Tinea unguim
T.versicolor	-	Tinea versicolor
T.capitis	-	Tinea capitis
T.barbae	-	Tinea barbae
T.cruris	-	Tinea cruris

T.mannum	-	Tinea mannum
T.fasciea	-	Tinea fasciea
NG	-	No Growth
T.rubrum	-	Trichophyton rubrum
T.verrucosum	-	Trichophyton verrucosum
T.mentagrophytes	-	Trichophyton mentagrophytes
T.tonsurans	-	Trichophyton tonsurans
M.furfur	-	Microsporum furfur
M.gypseum	-	Microsporum gypseum
E.flocossum	-	Epidermophyton flocossum

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S.NO	AGE	SEX	OP NUMBER	SPECIMEN	OCCUPATION	RISK FACTORS	ZCLINICAL DIAGNOSIS	KOH	Culture	DTM	MIC Griseo	MICKeto	MIC Itra	MIC Fluco
1	36	M	10548	Skin	Clerk	Diabetics	T.corporis	Negative	NG	Negative				
2	52	M	10732	Skin	Agriculturer	Animal Contacts	T.pedis	Negative	NG	Negative				
3	34	M	10629	Skin	Driver	Animal Contacts	T.corporis	Negative	NG	Negative				
4	29	F	10673	Nail	House wife	Close family contacts	T.unguium	Positive	T.rubrum	positive	0.06	0.06	0.03	1
5	8	F	10439	Skin	Student	Animal Contacts	T.pedis	Negative	NG	Negative				
6	34	M	10413	Skin	Agriculturer		T.pedis	Negative	NG	Negative				
7	33	M	10422	Skin	Vendor	Close family contacts	T.pedis	Negative	NG	Negative				
8	10	F	10406	Skin	Student	Close family contacts	T.corporis + T.verscicolor	Negative	NG	Negative				
9	34	M	10463	Skin	Lab Tech	Hyperhidrosis	T.corporis	Negative	NG	Negative				
10	45	F	10517	Skin	Agriculturer	Animal Contacts	T.corporis	Negative	NG	Negative				
11	30	F	10479	Skin	Agriculturer		T.corporis	Negative	NG	Negative				
12	18	M	10294	Skin	Student	Hostler	T.capitis	Negative	NG	Negative				
13	39	F	10925	Skin	House wife		T.barbae	Negative	NG	Negative				
14	10	M	10483	Hair	Student	Animal Contacts	T.capitis	Negative	NG	Negative				
15	29	M	12954	Skin	Salesman	Hyperhidrosis	T.corporis	Negative	NG	Negative				
16	58	M	12949	Skin	Agriculturer	Animal Contacts	T.corporis	Negative	NG	Negative				
17	72	M	12465	Skin	Agriculturer	Diabetics	T.pedis	Negative	NG	Negative				

18	58	F	12052	Skin	Agriculturer	Diabetics	T.cruris+ T.corporis	Negative	NG	Negative				
19	10	F	13122	Hair	Student		T.capitis	Negative	NG	Negative				
20	16	M	13619	Skin	Student	Hostler	T.corporis	Negative	NG	Negative				
21	42	M	13873	Skin	Constable	Diabetics	T.corporis	Negative	NG	Negative				
22	37	M	13229	Skin	Teacher	Close family contacts	T.corporis	Negative	NG	Negative				
23	12	M	14069	Skin	Student	Hostler	T.corporis	Negative	NG	Negative				
24	35	M	14068	Skin	Driver		T.corporis	Negative	NG	Negative				
25	29	F	16340	Skin	Agriculturer	Animal Contacts	T.corporis	Negative	NG	Negative				
26	65	F	14441	Nail	Agriculturer	Diabetics	T.unguium	Negative	NG	Negative				
27	3	M	15992	Hair			T.capitis	Negative	NG	Negative				
28	52	M	13844	Nail	Agriculturer	Close family contacts	T.unguium	Negative	NG	Negative				
29	59	M	14215	Nail	waiter	Hyperhidrosis	T.unguium	Negative	NG	Negative				
30	38	F	17493	Skin	Anganwadi worker	Animal Contacts	T.corporis	Negative	NG	Negative				
31	8	F	10439	Skin	Student	Animal Contacts	T.corporis	Negative	NG	Negative				
32	33	M	10422	Skin	House wife		T.corporis	Negative	NG	Negative				
33	27	M	15262	Skin	clerk		T.corporis	Negative	NG	Negative				
34	49	F	13823	Skin	House wife		T.corporis	Negative	NG	Negative				
35	10	F	13283	Skin	Student	Close family contacts	T.corporis	Negative	NG	Negative				

36	30	F	13294	Skin	Banking	Diabetics	T.corporis	Positive	T.rubrum	positive	0.12	0.12	0.12	1
37	22	M	13843	Skin	Student	Hostler	T.corporis	Negative	NG	Negative				
38	13	F	14639	Skin	Student	Animal Contacts	T.cruris	Negative	NG	Negative				
39	68	M	16588	Skin	loadman	Diabetics	T.corporis	Negative	NG	Negative				
40	17	F	17583	Skin	Supervisor		T.corporis	Negative	NG	Negative				
41	16	M	17573	Skin	Student	Hostler	T.corporis+ T.cruris	Negative	NG	Negative				
42	15	F	17345	Skin	Student	Hostler	T.corporis+ T.mannum	Positive	T.rubrum	positive	0.25	0.25	0.06	2
43	18	M	17828	Skin	Student	Hostler	T.corporis+ T.fasciei	Positive	T.rubrum	positive	0.12	0.5	0.12	1
44	28	M	17848	Skin	Constable	Hyperhidrosis	T.corporis+ T.cruris+ T.unguium	Positive	T.rubrum	positive	0.25	0.12	0.06	1
45	43	M	16345	Skin	Driver	Animal Contacts	T.corporis + T.cruris	Positive	T.rubrum	positive	0.06	0.12	0.12	1
46	10	M	16982	Skin	student	Animal Contacts	T.corporis	Negative	NG	Negative				
47	21	M	18003	Skin	Electrician		T.corporis	Positive	T.rubrum	positive	0.25	0.12	0.06	4
48	16	M	17995	Skin	Student	Hostler	T.corporis+ T.cruris	Positive	T.rubrum	positive	0.25	0.12	0.06	1
49	50	M	27633	Skin	Agriculturer	Animal Contacts	T.corporis + T.cruris	Positive	T.rubrum	positive	0.06	0.06	0.06	1

50	35	M	12774	Skin	Vendor		T.corporis	Positive	T.rubrum	positive	0.12	0.12	0.12	1
51	16	M	12993	Skin	Student	Hostler	T.corporis	Positive	T.rubrum	positive	0.25	0.5	0.06	2
52	14	M	16432	Skin	Student	Hostler	T.corporis	Positive	T.rubrum	positive	0.25	0.12	0.12	1
53	26	F	16433	skin	House wife		T.corporis	Negative	NG	Negative				
54	50	M	14832	Skin	Watchman	Diabetics	T.corporis	Positive	T.rubrum	positive	0.25	0.25	0.06	2
55	12	F	23841	Skin	Student		T.corporis	Negative	NG	Negative				
56	75	F	13423	Skin	housewife	Diabetics	T.corporis+ T.cruris	Positive	T.rubrum	positive	0.06	0.12	0.03	1
57	39	M	18433	Skin	house wife		T.corporis + T.cruris	Positive	T.rubrum	positive	0.25	0.25	0.06	4
58	15	M	18523	Skin	Student	Hostler	T.corporis	Positive	T.rubrum	positive	0.12	0.06	0.12	1
59	40	F	17334	Nail	Vertinary hospital worker	Animal Contacts	T.unguium	Negative	T.verrucosum	positive	0.25	0.5	0.25	4
60	63	F	18532	Skin	housewife	Diabetics	T.corporis	Positive	T.rubrum	positive	0.25	0.12	0.06	1
61	19	F	17465	Skin	Nurse	Animal Contacts	T.corporis+ T.cruris+ T.mannum	Negative	Scopulariopsis	Negative				
62	38	M	18551	Skin	Teacher		T.corporis	Positive	T.rubrum	positive	0.06	0.12	0.06	1
63	60	F	18349	Skin	housewife	Diabetics	T.corporis	Positive	T.rubrum	positive	0.25	0.12	0.25	2
64	50	M	17340	Skin	Driver	Diabetics	T.corporis	Positive	T.rubrum	positive	0.25	0.12	0.06	1
65	45	M	16341	Skin	clerk	Diabetics	T.corporis	Positive	NG	Negative				

66	45	M	18675	Skin	Cleaner	Diabetics	T.corporis	Positive	T.rubrum	positive	0.25	0.25	0.12	1
67	15	M	17593	Skin	Student	Hostler	T.corporis	Positive	T.rubrum	positive	0.25	0.12	0.06	2
68	28	F	17483	Skin	House wife		T.mannum	Negative	NG	Negative				
69	9	M	18459	Skin	Student	Close family contacts	T.pedis	Negative	NG	Negative				
70	27	F	17683	Skin	Banking	Close family contacts	T.corporis+ T.unguium	Negative	NG	Negative				
71	34	F	19275	Skin	Sweeper	Close family contacts	T.pedis	Negative	NG	Negative				
72	50	M	19473	Skin	Vendor	Close family contacts	T.corporis	Positive	T.rubrum	positive	0.06	0.12	0.03	1
73	28	F	19783	Skin	House wife		T.corporis	Positive	T.rubrum	positive	0.25	0.12	0.12	2
74	26	F	19530	Skin	House wife		T.corporis	Positive	T.rubrum	positive	0.12	0.5	0.12	1
75	35	F	19533	Skin	Babysitter		T.corporis	Positive	T.rubrum	positive	0.25	0.12	0.06	2
76	24	M	19351	Skin	Salesman		T.cruris	Negative	NG	Negative				
77	53	F	13453	Skin	housewife	Diabetics	T.corporis+ T.unguium	Negative	NG	Negative				
78	5	F	14367	Skin	Student	Close family contacts	T.fasciei	Positive	T.rubrum	positive	0.25	0.12	0.25	1
79	25	M	15467	Skin	Constable	Hyperhidrosis	T.fasciei	Negative	NG	Negative				
80	45	M	13478	Skin	Driver	Animal Contacts	T.corporis	Negative	NG	Negative				
81	22	F	14567	Skin	Nurse		T.corporis	Positive	T.rubrum	positive	0.06	0.06	0.12	1
82	56	F	15632	Skin	housewife	Diabetics	T.corporis	Negative	NG	Negative				

83	29	F	14332	Skin	Banking	Close family contacts	T.corporis+ T.unguium	Negative	NG	Negative				
84	37	F	14555	Skin	Teacher		T.pedis	Negative	NG	Negative				
85	34	M	17841	Skin	Teacher		T.corporis	Negative	NG	Negative				
86	45	M	18756	Skin	hospital worker	Close family contacts	T.corporis	Negative	NG	Negative				
87	40	M	17845	Skin	Driver		T.corporis	Negative	NG	Negative				
88	34	M	18765	Skin	Teacher		T.corporis	Positive	NG	Negative				
89	54	F	13456	Nail	housewife	Diabetics	T.unguium+ T.pedis	Positive	T.verrucosum	positive	0.12	0.12	0.03	2
90	50	F	14356	Skin	housewife		T.fasciei	Negative	NG	Negative				
91	38	M	14560	Skin	Vendor		T.corporis	Positive	T.rubrum	positive	0.25	0.12	0.06	2
92	40	M	18769	Nail	Driver		T.unguium	Positive	T.verrucosum	positive	0.25	0.5	0.06	2
93	15	F	18743	Skin	Student	Hostler	T.corporis	Positive	T.mentagrophytes	positive	0.12	0.12	0.015	2
94	65	F	14571	Skin	housewife	Diabetics	T.corporis	Negative	NG	Negative				
95	45	M	16785	Skin	driver	Diabetics	T.corporis	Positive	T.rubrum	positive	0.12	0.12	0.12	1
96	49	M	17865	Skin	clerk	Diabetics	T.corporis	Positive	T.rubrum	positive	0.25	0.25	0.06	1
97	45	M	18756	Skin	Agriculturer	Diabetics	T.corporis	Positive	T.verrucosum	positive	0.12	0.06	0.06	2
98	52	M	18743	Nail	Agriculturer	Close family contacts	T.unguium	Negative	NG	Negative				
99	33	M	17654	Skin	Agriculturer		T.corporis	Positive	T.rubrum	positive	0.25	0.12	0.12	1
100	24	M	14642	Skin	Auto Driver		T.pedis	Positive	T.verrucosum	positive	0.06	0.06	0.06	2

101	26	M	15643	Nail	Bus Driver	Hyperhidrosis	T.unguium	Negative	NG	Negative				
102	36	F	17654	Skin	Agriculturer	Animal Contacts	T.corporis	Positive	T.rubrum	positive	0.06	0.12	0.03	1
103	10	M	16453	Hair	Student		T.capitis /kerion	Positive	NG	Negative				
104	43	F	17465	Skin	Agriculturer	Diabetics	T.pedis	Negative	NG	Negative				
105	50	M	17685	Skin	Agriculturer	Diabetics	T.corporis+ T.cruris	Positive	NG	Negative				
106	20	F	14672	Nail	Nurse	Animal Contacts	T.unguium+ T.pedis	Positive	NG	Negative				
107	45	M	16759	Skin	Agriculturer	Close family contacts	T.corporis	Positive	NG	Negative				
108	56	F	17564	Skin	housewife		T.corporis	Positive	NG	Negative				
109	13	M	18753	Skin	Student	Animal Contacts	T.corporis	Positive	T.rubrum	positive	0.25	0.12	0.06	1
110	19	M	15648	Skin	Student	Hostler	T.corporis	Positive	NG	Negative				
111	21	M	19860	Skin	Student	Hostler	T.corporis	Positive	T.rubrum	positive	0.25	0.12	0.25	2
112	19	F	18551	Skin	Student	Hostler	T.corporis	Positive	T.rubrum	positive	0.12	0.12	0.12	1
113	30	M	17563	Nail	Mechanic		T.unguium+ T.pedis	Negative	Fusarium	Negative				
114	50	F	18672	Hair	Agriculturer	Diabetics	T.capitis	Negative	NG	Negative				
115	42	M	12852	Nail	Agriculturer	Animal Contacts	T.unguium	Negative	NG	Negative				
116	77	M	15326	Nail	Agriculturer	Diabetics	T.unguium	Negative	NG	Negative				
117	24	M	15372	Nail	Salesman		T.unguium	Positive	T.rubrum	positive	0.25	0.12	0.12	2
118	50	F	19555	Hair	Agriculturer		T.capitis	Negative	NG	Negative				

119	36	M	15657	Nail	Vendor		T.unguium	Negative	NG	Negative				
120	33	M	18632	Nail	Clerk	Animal Contacts	T.unguium	Negative	NG	Negative				
121	37	F	971	Nail	Mason		T.unguium	Negative	NG	Negative				
122	20	M	911	Nail	Electrician	Animal Contacts	T.unguium	Negative	NG	Negative				
123	60	F	916	Hair	Agriculturer	Diabetics	T.capitis	Negative	NG	Negative				
124	24	M	907	Nail	Clerk	Animal Contacts	T.unguium	Negative	T.rubrum	positive	0.25	0.25	0.06	1
125	20	M	1183	Hair	Watchman	Animal Contacts	T.capitis	Negative	NG	Negative				
126	21	M	1187	Nail	Student	Hostler	T.unguium	Negative	NG	Negative				
127	26	M	1637	Nail	Driver	Animal Contacts	T.unguium	Negative	NG	Negative				
128	51	M	22971	Nail	Agriculturer	Animal Contacts	T.unguium	Negative	NG	Negative				
129	36	M	4432	Nail	clerk	Animal Contacts	T.unguium	Negative	NG	Negative				
130	23	M	4551	Nail	Mechanic		T.unguium	Negative	NG	Negative				
131	50	F	2965	Skin	Agriculturer	Diabetics	T.mannum	Positive	T.mentagrophytes	positive	0.25	0.25	0.12	8
132	28	F	1436	Nail	Babysitter	Close family contacts	T.unguium	Negative	NG	Negative				
133	31	F	4736	Nail	Cleaner	Close family contacts	T.unguium	Negative	NG	Negative				
134	32	M	4856	Hair	Housekeeping		T.capitis	Negative	NG	Negative				
135	45	M	4935	Skin	cook	Diabetics	T.corporis	Positive	T.rubrum	positive	0.25	0.12	0.12	2
136	30	M	4937	Nail	Vendor		T.unguium	Negative		Negative				
137	45	M	4982	Skin	Agriculturer	Close family contacts	T.corporis	Positive	T.rubrum	positive	0.25	0.25	0.25	1

138	53	M	5079	Nail	Agriculturer		T.unguium	Negative		Negative				
139	30	M	5081	Hair	Constable	Hyperhidrosis	T.capitis	Negative		Negative				
140	16	F	5183	Hair	Student	Hostler	T.capitis	Negative		Negative				
141	6	F	5276	Hair	Student	Animal Contacts	T.capitis	Negative		Negative				
142	18	M	5400	Skin	Student	Hostler	T.corporis	Positive	T.rubrum	positive	0.12	0.12	0.12	1
143	10	M	5582	Hair	Student	Animal	T.capitis	Positive	T.tonsurans	positive	0.25	0.06	0.06	2
144	25	M	6432	Nail	Driver	Contacts	T.unguium	Negative		Negative				
145	28	M	5475	Nail	Sweeper		T.unguium	Negative		Negative				
146	18	F	1588	Hair	Student	Hostler	T.capitis	Negative		Negative				
147	41	M	20112	Skin	Sweeper	Diabetics	T.corporis	Positive	T.rubrum	positive	0.25	0.12	0.06	1
148	45	M	5513	Nail	cook	Diabetics	T.unguium	Negative		Negative				
149	66	M	2364	Nail	Agriculturer	Diabetics	T.unguium	Negative		Negative				
150	8	F	11941	Hair	Student	Close family contacts	T.capitis	Negative		Negative				
151	55	F	19194	Nail	Housewife		T.unguium	Negative		Negative				
152	27	M	5912	Nail	Lab Tech		T.unguium	Negative		Negative				
153	20	M	5439	Nail	Watchman	Animal Contacts	T.unguium	Negative		Negative				
154	2	M	1609	Skin		Close family contacts	T.corporis	Negative		Negative				
155	30	F	1303	Nail	Sweeper	Animal Contacts	T.unguium	Positive	E.floccosum	positive	0.06	0.06	0.03	4

156	26	M	2011	Nail	Teacher		T.unguium	Negative		Negative				
157	14	M	6432	Hair	Student	Hostler	T.capitis	Negative		Negative				
158	26	M	5865	Nail	Lab Tech	Animal Contacts	T.unguium	Negative		Negative				
159	48	M	7410	Nail	Agriculturer	Animal Contacts	T.unguium	Negative		Negative				
160	23	F	7425	Skin	Teacher		T.corporis	Negative	T.rubrum	positive	0.25	0.12	0.12	4
161	15	M	7673	Skin	Student	Hostler	T.corporis	Positive	T.rubrum	positive	0.5	0.5	0.06	1
162	20	F	7636	Nail	Babysitter	Animal Contacts	T.unguium	Positive	T.rubrum	positive	0.25	0.12	0.06	1
163	28	M	7647	Hair	Clerk	TB	T.capitis	Negative		Negative				
164	19	F	7909	Skin	Student	Hostler	T.corporis	Positive	T.rubrum	positive	0.25	0.25	0.12	2
165	52	M	7911	Nail	Watchman	Diabetics	T.unguium	Negative		Negative				
166	50	M	7801	Skin	Supervisor	Diabetics	T.corporis	Positive	T.rubrum	positive	0.25	0.25	0.06	2
167	35	M	7995	Nail	Flower seller	Close family contacts	T.unguium	Negative	NG	Negative				
168	69	M	7984	Skin	Supervisor	Diabetics	T.corporis	Positive	T.rubrum	positive	0.25	0.25	0.06	1
169	38	F	8094	Nail	Lab Tech		T.unguium	Negative	NG	Negative				
170	35	M	8103	Skin	Vendor		T.corporis	Positive	T.rubrum	positive	0.12	0.12	0.12	1
171	45	M	9720	Skin	Teacher	Diabetics	T.corporis	Positive	T.rubrum	positive	0.25	0.12	0.25	2
172	38	F	9666	Skin	Babysitter	Diabetics	T.corporis+ T.verscicolor	Positive	M.Furfur	Negative				
173	33	F	9667	Nail	Teacher		T.unguium	Negative	NG	Negative				
174	46	M	9643	Nail	cook	Close family contacts	T.unguium	Negative	NG	Negative				

175	19	F	8149	Nail	Nurse		T.unguium	Negative	NG	Negative				
176	26	M	8116	Skin	Auto Driver	TB	T.corporis	Positive	T.rubrum	positive	0.25	0.25	0.12	1
177	46	M	8127	Hair	Vendor	Diabetics	T.capitis	Negative	NG	Negative				
178	49	M	8130	Skin	Sweeper	Close family contacts	T.corporis	Positive	T.rubrum	positive	0.25	0.12	0.06	1
179	36	M	8133	Skin	Clerk	Close family contacts	T.corporis	Positive	T.rubrum	positive	0.5	0.12	0.06	1
180	51	F	8341	Skin	Housewife	Close family contacts	T.corporis	Positive	T.rubrum	positive	0.25	0.12	0.25	1
181	30	F	8368	Skin	Teacher		T.corporis	Positive	T.rubrum	positive	0.25	0.25	0.12	2
182	57	M	831	Skin	dhobi	Diabetics	T.corporis	Positive	T.rubrum	positive	0.25	0.25	0.06	1
183	28	M	8346	Nail	Mechanic		T.unguium	Negative	NG	Negative				
184	31	F	8340	Hair	Vendor		T.capitis	Positive	M.gypseum	positive	0.06	0.12	0.06	4
185	37	M	8409	Skin	Electrician	Diabetics	T.corporis	Positive	T.rubrum	positive	0.25	0.12	0.06	2
186	24	F	8415	Nail	Teacher	Animal Contacts	T.unguium	Positive	T.rubrum	positive	0.12	0.12	0.12	1
187	39	F	8439	Skin	Housekeeping		T.corporis	Positive	T.rubrum	positive	0.25	0.5	0.25	2
188	53	F	8548	Skin	Agriculturer		T.corporis	Positive	NG	Negative				
189	38	F	8562	Nail	Vendor	Close family contacts	T.unguium	Positive	T.mentagrophytes	positive	0.06	0.06	0.015	2
190	27	M	8583	Skin	Electrician		T.corporis	Positive	NG	Negative				
191	34	F	8571	Skin	Agriculturer	Close family contacts	T.corporis	Positive	NG	Negative				
192	33	F	8735	Nail	Flower seller	Close family contacts	T.unguium	Positive	T.mentagrophytes	positive	0.25	0.25	0.12	4

193	58	M	8731	Skin	Watchman	Diabetics	T.corporis	Positive	NG	Negative				
194	58	F	8780	Skin	Housewife	Close family contacts	T.corporis	Positive	T.mentagrophytes	positive	0.06	0.03	0.03	2
195	57	M	8842	Skin	Agriculturer	Diabetics	T.corporis	Positive	NG	Negative				
196	35	M	8855	Skin	Lab Tech		T.corporis	Positive	T.mentagrophytes	positive	0.03	0.12	0.015	2
197	30	F	8861	Nail	banking	Animal Contacts	T.unguium	Positive	NG	Negative				
198	28	M	8889	Nail	Electrician		T.unguium	Positive	NG	Negative				
199	32	F	9335	Nail	Sweeper		T.unguium	Positive	NG	Negative				
200	23	M	9320	Skin	Vendor	Close family contacts	T.corporis	Positive	T.rubrum	positive	0.25	0.12	0.06	1
201	26	M	13893	Skin	Mason	Animal Contacts	T.corporis	Positive	T.rubrum	positive	0.25	0.12	0.25	1
202	27	M	13892	Skin	Clerk		T.cruris+ T.corporis	Positive	E.floccosum	positive	0.25	0.5	0.06	4
203	55	F	13761	Nail	Agriculturer	Diabetics	T.unguium	Positive	NG	Negative				
204	21	F	13891	Skin	Nurse		T.corporis	Positive	T.rubrum	positive	0.25	0.25	0.06	2
205	27	F	13609	Skin	Housewife	Close family contacts	T.unguium	Negative	NG	Negative				
206	30	M	13976	Skin	conductor	Close family contacts	T.capitis+ T.corporis	Positive	NG	Negative				
207	45	F	13986	Nail	Agriculturer	Diabetics	T.unguium	Negative	NG	Negative				
208	55	M	13750	Hair	Agriculturer	Animal Contacts	T.corporis+ T.capitis	Positive	T.rubrum	positive	0.12	0.5	0.03	1
209	20	M	13580	Hair	Student	Hostler	T.capitis	Positive	NG	Negative				

210	22	M	13905	Skin	Electrician	Animal Contacts	T.corporis	Negative	NG	Negative				
211	35	M	13506	Skin	Constable		T.corporis	Negative	NG	Negative				
212	26	M	13509	Nail	Watchman		T.mannum + T.unguium	Positive	T.verrucosum	positive	0.25	0.5	0.25	4
213	12	M	13256	Skin	Student	Hostler	T.capitis	Negative	NG	Negative				
214	43	F	13245	Skin	Flower seller	Diabetics	T.corporis	Positive	NG	Negative				
215	32	M	13204	Hair	cook	Close family contacts	T.capitis	Negative	NG	Negative				
216	42	M	14444	Skin	Mechanic	Animal Contacts	T.corporis	Negative	NG	Negative				
217	28	F	14443	skin	Teacher	Animal Contacts	T.corporis	Positive	T.mentagrophytes	positive	0.25	0.25	0.03	2